HPLC METHODS FOR PHARMACEUTICAL ANALYSIS

Volumes 2-4

George Lunn

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ACKNOWLEDGMENTS

These days the production of a technical book is a complex process requiring the talents of many people. Even by modern standards, however, this was a particularly involved process. The raw data initially consisted of thousands of individual files, one for each abstract. These files were converted into a database that was used to make the CD for the electronic version as well as prepare the coded report that was used to set the galley and page proofs. The people who worked on this project are as follows:

For Synexchem Consulting Services International LLC: Software Developers Peter Baričič and Martin Mackov; Abstracters Elena Belajová, Research Institute of Foods; Alica Čániová, Slovak Technical University, Department of Analytical Chemistry; Katarina Hroboňová, Slovak Technical University, Department of Analytical Chemistry; Gabriela Kristofiková; Pavol Kubalec, Slovak Technical University, Department of Analytical Chemistry; Gabriela Machalová; Martina Nahálková; Mária Tegzová, Drug Research Institute; and Pavol Valachovič, Drug Research Institute

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The use of the National Library of Medicine, National Institutes of Health Library, and the FDA Medical Library is greatly appreciated. Although many people have helped with the preparation of this work, the mistakes are my own. I would appreciate hearing from anyone who has corrections, comments, or suggestions. I can be reached at lunng@cder.fda.gov.

The content of this volume does not necessarily reflect the views or policies of the Food and Drug Administration, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

ABOUT THIS BOOK

SCOPE

A computer search was used to identify relevant references. The search was conducted using Medline, 1980 to date. Since references from the *Journal of Liquid Chromatography* are not included in Medline, these references were manually added to the database.

The search strategy was as follows: HPLC (tw) **or** HPLC (mh) **or** liquid chromatography (mh) **and** USAN drug name (tw or mh), where tw = text word and mh = MESH heading.

In addition to the Medline search, some journals were routinely surveyed for relevant articles. These journals were:

American Journal of Health-System Pharmacy
Analyst
Analytical Chemistry
Antimicrobial Agents and Chemotherapy
Arzneimittelforschung
Biological and Pharmaceutical Bulletin
Biomedical Chromatography
Biopharmaceutics and Drug Disposition
Chemical and Pharmaceutical Bulletin
Chromatographia
Clinical Chemistry
Clinical Pharmacology and Therapeutics
Drug Metabolism and Disposition
Farmaco
Journal of Analytical Toxicology
Journal of AOAC International
Journal of Chromatographic Science
Journal of Chromatography, Part A and Part B
Journal of Clinical Pharmacology
Journal of Forensic Sciences

Journal of Liquid Chromatography and Related Technology
Journal of Medicinal Chemistry
Journal of Pharmaceutical and Biomedical Analysis
Journal of Pharmaceutical Sciences
Journal of Pharmacology and Experimental Therapeutics
Pharmaceutical Research
Pharmazie
Therapeutic Drug Monitoring
Xenobiotica

Many other journals were consulted when relevant articles were identified by computer searches.

CHAPTER STRUCTURE

Each chapter is headed by the name and structure of the target compound as well as other useful data such as the CAS Registry Number, molecular formula, and molecular weight. In general the United States Adopted Name (USAN) is used throughout to identify each drug, although we sometimes use a truncated version of this name, e.g., naproxen for naproxen sodium. Exceptions are clavulanate potassium, which is listed in the "Clavulanic Acid" monograph, EDTA, which is used in preference to edetic acid, hyoscyamine, which is listed in the monograph for its racemate atropine, isotretinoin and tretinoin, which are listed in the monograph "Retinoic Acid," levonorgestrel, which is listed in the "Norgestrel" monograph, and some of the steroids, which are listed for convenience in the monograph "Estrogens, conjugated." Names of derivatives, such as esters, which would have different chromatographic properties, are identified by placing the derivative name in parentheses after the retention time.

Reference is also made at the head of each chapter to the relevant abstract in the *Merck Index*. **Note that these numbers refer to the 12th edition of the Merck Index.**[1] Much useful information, such as melting point, solubility, optical rotation, and references to reviews, can be found in the *Merck Index*. In addition, the relevant sections in the series *Organic Chemistry of Drug Synthesis*, by Lednicer and Mitscher,[2–6] are referenced. These books give valuable information about the syntheses of various drugs and this may be helpful in determining impurities, understanding degradation reactions, and so on.

ABSTRACT STRUCTURE

The detailed procedures given normally contain the following sections. Of course, not all papers give full details, so some sections may be missing.

Matrix
Sample Preparation
Guard Column
Column
Mobile Phase
Flow Rate

Injection Volume	Also
Retention Time	Noninterfering
Detector	Interfering
Internal Standard	Limit of Detection
Column Temperature	Limit of Quantitation
Extracted	Key words
Simultaneous	Reference

ABSTRACT CONVENTIONS

Also	Compounds that can be analyzed at the same time. It is not specified whether they interfere, but they can be extracted. See also Extracted, Simultaneous.
Column	Dimensions are length (mm) × internal diameter (mm), and the material is stainless steel unless otherwise indicated.
Column Temperature	If other than ambient (all temperatures are in degrees C)
Derivatization	Pre-column unless otherwise mentioned (in Key Words)
Detector	Wavelengths in nm
Extracted	Compounds that can be extracted from the matrix in question and analyzed at the same time and do not interfere. See also Also, Simultaneous.
Flow Rates	In mL/min.
Guard Column	Dimensions are length (mm) × internal diameter (mm)
Impurities	If method resolves compound and impurities (in Key Words)
Injection Volume	In microliters (μL). Injection Volume may be either the volume actually injected or the volume of the injection loop. If it is the volume actually injected, this value is also given in the Sample Preparation section. If the actual injection volume is not given in the Sample Preparation section, the Injection Volume given is that of the injection loop.
Interfering	Compounds that interfere with the analysis of the target compound. Compounds which interfere with the chromatography of the internal standard (IS) are listed under simultaneous (another IS can always be selected or an external standard procedure can be used).
Matrix	A controlled vocabulary is used (see below)
Metabolites	If method resolves compound and metabolites (in Key Words)
Mobile Phase	Ratios are v/v and gradients are linear unless otherwise noted. Times given when describing gradient elution, and other procedures such as column switching, are the times for each step, e.g., "MeOH:water 15:85 for 4 min, to 50:50 over 2 min, maintain at 50:50 for 4 min." If we were to include the cumulative times (t) in the example above, it would read: "MeOH:water 15:85 for 4 min (t = 4), to 50:50 over 2 min (t = 6), maintain at 50:50 for 4 min (t = 10)."
Noninterfering	Compounds which do not interfere with the analysis for various reasons, e.g., they are not extracted, they are not detected.

Retention Time	This is frequently estimated from a reproduced chromatogram and so the accuracy may not be great (in minutes).
Simultaneous	Compounds which can be analyzed at the same time and do not interfere. Note that the compound cannot necessarily be extracted from the matrix in question (although it may be). See also Also, Extracted.
SPE	For the sake of consistency, conditioning procedures for solid-phase extraction (SPE) cartridges are always described at the beginning of the sample preparation sections. Bear in mind, however, that the conditioning procedure should be carried out just prior to use. Thus, if sample preparation is a lengthy procedure, it may be necessary to delay SPE cartridge conditioning until the step requiring the cartridge.
Species	If other than human; noun is used instead of adjective, e.g., cow not bovine. In some cases, human may be specified. For example, if **both** human blood and rat blood are analyzed, **both** human and rat will be indicated (in Key Words).

MATRIX

To help with searching, a controlled vocabulary is used to limit the number of terms in the matrix section. For example, the term raw materials is not used, the term bulk is used instead. In a number of cases the matrix is associated with various key words which can be used to narrow the search. For example, the matrix term blood has the key words plasma, serum, and whole blood associated with it. Thus, if you are interested in the determination of the drug in blood in general you should search the matrix field for blood. If, however, you are specifically interested in finding the drug in plasma you should search the key words field for plasma.

Matrix	Associated Key Words
bile	
blood	plasma, serum, whole blood
bulk	
CSF	
formulations	capsules, creams, injections, ointment, tablets, etc.
microsomal incubations	
milk	
perfusate	
reaction mixtures	
saliva	
solutions	buffer, water
tissue	brain, heart, kidney, liver, muscle, spleen, etc.
urine	

ABBREVIATIONS

BHT	2,6-di-tert-butyl-4-methylphenol, butylated hydroxytoluene

CE	capillary electrophoresis
DMSO	dimethyl sulfoxide
E	electrochemical detection
em	emission wavelength
EtOH	ethanol
ex	excitation wavelength
F	fluorescence detection
FW	formula weight
GPC	gel permeation chromatography
h	hour
HPLC	high-performance liquid chromatography
IS	internal standard
L	liter
LOD	limit of detection or some other description indicating that this is the smallest concentration or quantity that can be detected or analyzed for
LOQ	lower limit of quantitation, either given as such in the paper or taken as the lower limit of the linear quantitation range
M	molar (i.e., moles/L)
MeCN	acetonitrile
MeOH	methanol
min	minutes
mL	milliliter
mM	milli-molar (i.e., milli-moles/L)
MTBE	methyl tert-butyl ether
nM	nano-molar (i.e., nano-moles/L)
psi	pounds/sq. in. (1 psi = 6.89476 kPa)
s	seconds
SEC	size exclusion chromatography
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIM	selected-ion monitoring
SPE	solid phase extraction
Temp	temperature
U	units
UV	ultraviolet detection

WORKING PRACTICES

In general, good working practice, e.g., using high-grade materials, is assumed. Solutions containing compounds should be protected from light and silanized glassware should be used unless you have good reason to believe that these precautions are not necessary. Details of solution preparation are generally not given. It should be remembered that the preparation of a dilute aqueous solution of a relatively water-insoluble compound can frequently be made by dissolving the compound in a small volume of a water-miscible organic solvent and diluting this solution with water. A number of excellent texts[7–9] discuss good working practices and procedures in HPLC and these should be consulted.

It is also assumed that safe working practices are observed. Organic solvents should only be evaporated in a properly functioning chemical fume hood, correct protective equipment should be worn when dealing with potentially hazardous biological materials, and waste solutions should be disposed of in accordance with all applicable regulations.

A number of solvents are particularly hazardous. For example, benzene is a human carcinogen[10]; chloroform,[11] dichloromethane,[12] dioxane,[13] and carbon tetrachloride[14] are carcinogenic in experimental animals; and DMF[15] and MTBE[16,17] may be carcinogenic. Organic solvents are, in general, flammable and toxic by inhalation, ingestion, and skin absorption. Sodium azide is carcinogenic and toxic and liberates explosive, volatile, toxic hydrazoic acid with acid. Sodium azide can form explosive heavy metal azides, e.g., with plumbing fixtures, and so should not be discharged down the drain.[18] Disposal procedures have been described for a number of hazardous drugs and reagents[18] and recent papers describe a procedure for the hydrolysis of acetonitrile in waste solvent to the much less toxic acetic acid and ammonia.[19,20] Recent work has shown that n-hexane is surprisingly toxic.[21]

PIC REAGENTS

These reagents are offered by Waters as buffered solutions containing the following compounds:

PIC A is tetrabutylammonium sulfate
PIC B5 is pentanesulfonic acid
PIC B6 is hexanesulfonic acid
PIC B7 is heptanesulfonic acid
PIC B8 is 1-octanesulfonic acid
PIC D4 is dibutylamine phosphate

SUPPLIERS

Suppliers of critical items such as columns are given in the abstracts but the suppliers for widely available items are not listed. These suppliers are as follows:

Item	Supplier
Adsorbosphere	Alltech Associates
Asahipak	Asahi Chemical
Bakerbond	J.T. Baker
Bond Elut	Varian
μBondapak	Waters
Chiralcel	Daicel
Co:Pell	Whatman
Corasil	Waters
Cyclobond	Advanced Separation Technologies
Econosil	Alltech Associates
Econosphere	Alltech Associates

Extrelut	E. Merck
Hypersil	Shandon
Inertsil	MetaChem
LiChroprep	E. Merck
LiChrosorb	E. Merck
LiChrosphere	E. Merck
Micropak	Varian
Microsorb	Rainin
NewGuard	Applied Biosystems
Nova-Pak	Waters
Nucleosil	Macherey Nagel
Partisil	Whatman
Pecosphere	Perkin-Elmer
Porasil	Waters
Sep-Pak	Waters
Spheri-5	Applied Biosystems
Spheri-10	Applied Biosystems
Spherisorb	Phase Separations
SPICE	Analtech
Supelcosil	Supelco
Ultrasphere	Beckman
Ultremex	Phenomenex
Vydac	The Separations Group
Zorbax	Mac-Mod Analytical

This list is not intended to be definitive. Many other companies supply these pieces of equipment.

TRADEMARKS

The following trademarks are used:

Trademark	Company
Adsorbosphere	Alltech Associates, Inc.
Asahipak	Asahi Chemical Industry Co. Ltd.
Bakerbond	J.T. Baker
Bond Elut	Varian Associates, Inc.
μBondapak	Waters Associates, Inc.
Chiralcel	Daicel Chemical Industries, Ltd.
Co:Pell	Whatman Chemical Separation Co.
Corasil	Waters Associates, Inc.
Cyclobond	Advanced Separation Technologies, Inc.
Econosil	Alltech Associates, Inc.
Econosphere	Alltech Associates, Inc.
Extrelut	E. Merck
Hypersil	Shandon Scientific, Ltd.
Inertsil	GL Sciences Inc.
LiChroprep	E. Merck

Trademark	Company
LiChrosorb	E. Merck
LiChrosphere	E. Merck
Micropak	Varian Associates, Inc.
Microsorb	Rainin Instrument Co. Inc.
NewGuard	Applied Biosystems
Nova-Pak	Waters Associates, Inc.
Nucleosil	Macherey Nagel
Partisil	Whatman Chemical Separation Co.
Pecosphere	Perkin-Elmer
PIC	Waters Associates, Inc.
Porasil	Waters Associates, Inc.
Resolve	Waters Associates, Inc.
Sep-Pak	Waters Associates, Inc.
Spheri-5	Applied Biosystems
Spheri-10	Applied Biosystems
Spherisorb	Phase Separations, Ltd.
SPICE	Analtech
Supelcosil	Supelco, Inc.
Ultrasphere	Beckman Instruments, Inc.
Ultremex	Phenomenex, Inc.
Vydac	The Separations Group
Zorbax	DuPont Company

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6. Lednicer, D., *The Organic Chemistry of Drug Synthesis*, Vol. 5, Wiley, New York, 1995.
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12. Reference 15, pp. 2311–2312.

13. Reference 15, pp. 1449–1450.
14. Reference 15, pp. 701–702.
15. Reference 15, p. 1378.
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18. Lunn, G. and Sansone, E.B., *Destruction of Hazardous Chemicals in the Laboratory*, 2nd ed., Wiley, New York, 1994.
19. Gilomen, K., Stauffer, H.P., and Meyer, V.R., Detoxification of acetonitrile–water wastes from liquid chromatography. *Chromatographia* **41**, 488–491 (1995).
20. Gilomen, K., Stauffer, H.P., and Meyer, V.R., Management and detoxification of acetonitrile wastes from liquid chromatography. *LC.GC* **14**, 56–58 (1996).
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PREFACE

This book is a collection of procedures for the analysis of a number of pharmaceuticals using high-performance liquid chromatography (HPLC). This book is a continuation of *HPLC Methods for Pharmaceutical Analysis*, published in 1997. The previous book described analytical procedures for the 170 most commonly used drugs. This book lists procedures for the analysis of the remaining drugs that are used for both medical and veterinary purposes. In addition, a few recent procedures for the analysis of drugs covered in the first volume are described in this volume.

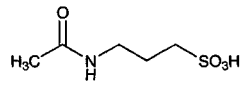
Despite the ready availability of laboratory-based literature searching, this resource is not exploited as much as it might be. One reason for this reluctance is, of course, that a computer search merely produces a listing of possibly relevant references. Tedious and time-consuming searches in the library are necessary to find the most relevant reference that can be turned into a practical analytical procedure in the searcher's own laboratory. The reference finally chosen will, naturally, depend on the individual circumstances, such as the matrix in which the drug is present and availability of equipment. This book circumvents this lengthy process by providing a number of abstracted and evaluated procedures for the analysis of each drug. The analyst can rapidly identify a relevant procedure and put it into practice.

In addition to the analytical matrix, other factors may be important when choosing an analytical procedure. Accordingly, we have noted such features of the analytical procedures as sensitivity, mode of detection, other compounds that interfere with the analysis, and other drugs that may be determined at the same time.

Readers familiar with our previous publications, *HPLC Methods for Pharmaceutical Analysis* (George Lunn and Norman R. Schmuff, John Wiley, New York, 1997) and *Handbook of Derivatization Reactions for HPLC* (George Lunn and Louise C. Hellwig, John Wiley, New York, 1998), will notice many similarities. The abstract structure is very similar and the philosophy that the procedures should be reproducible without reference to the original literature has been continued. Like the previous volumes, this book is also available on a CD in an electronic form. The software is new and hence the appearance on the computer screen is different but the ease of use has been retained. The CD contains all the data from the abovementioned pharmaceutical analysis book as well as the data from this volume, the data from the other two volumes of HPLC methods, and the volume of capillary electrophoresis methods that is being published simultaneously with this volume.

Contents

Acamprosate



Molecular formula: $C_5H_{11}NO_4S$
Molecular weight: 181.21
CAS Registry No.: 77337-76-9

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 2.97

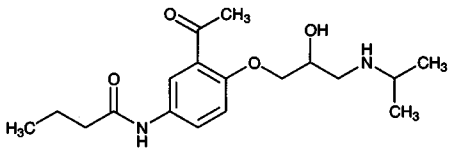
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acebutolol



Molecular formula: $C_{18}H_{28}N_2O_4$
Molecular weight: 336.43
CAS Registry No.: 37517-30-9
Merck Index: 16
Lednicer No.: 2 109

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 50 μL water + 500 μL MeCN, vortex for 1 min. Centrifuge at 6000 rpm for 10 min. Evaporate the supernatant to 200 μL at 40° under a stream of nitrogen, vortex for 30 s. Inject a 30-80 μL aliquot.

HPLC VARIABLES

Column: 100 × 8.0 4 μm Radial-pak Novapak C18
Mobile phase: MeCN:buffer 14:86 (Buffer was 2 g citric acid, 2 g sodium acetate, and 1 mL triethylamine in 1 L water.)
Flow rate: 2.5
Injection volume: 30-80
Detector: F ex 330 em 440

CHROMATOGRAM

Retention time: 7.4

OTHER SUBSTANCES

Extracted: norfloxacin, pefloxacin
Simultaneous: ciprofloxacin, lomefloxacin, ofloxacin

KEY WORDS

acebutolol is IS; serum

REFERENCE

Abanmi,N.; Zaghloud,I.; El Sayed,N.; al-Khamis,K.I. Determination of pefloxacin and its main active metabolite in human serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1996**, *18*, 158–163.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL 67 mM pH 7.4 phosphate buffer, 200 μL 1 M NaOH, and 6 mL MTBE to 1 mL plasma. Shake for 10 min, centrifuge at 1300 g at 4° for 5 min, freeze the aqueous layer in acetone/dry ice. Add 200 μL 10 mM HCl to the organic layer, shake for 10 min, centrifuge at 1300 g for 5 min. Freeze the aqueous layer, discard the organic phase, eliminate traces of the organic layer using a stream of cold air over the aqueous layer for 3-4 min. Thaw the aqueous layer, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb hexyl
Mobile phase: MeCN:15 mM pH 3.5 KH_2PO_4 containing 0.05% triethylamine 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 238

CHROMATOGRAM

Retention time: 3.3

Internal standard: acebutolol

OTHER SUBSTANCES
Extracted: celiprolol

KEY WORDS
plasma; acebutolol is IS

REFERENCE
Verbesselt,R.; Zugravu,A.; Tjandramaga,T.B.; De Schepper,P.J. Liquid chromatographic determination of total celiprolol or (S)-celiprolol and (R)-celiprolol simultaneously in human plasma, *J.Chromatogr.B*, **1996**, *683*, 231–236.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 1 M NaOH + 5 mL chloroform, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL 0.05% S-(+)-1-(1-naphthyl)ethylisocyanate in chloroform, vortex for 30 s, evaporate to dryness under reduced pressure, reconstitute with 200 μL chloroform, inject a 50-175 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 silica
Mobile phase: Hexane:chloroform:MeOH 60:38:2
Flow rate: 2
Injection volume: 50-175
Detector: F ex 220 em 345

CHROMATOGRAM
Retention time: 14.9 (R), 16.5 (S)
Internal standard: (±)-acebutolol

OTHER SUBSTANCES
Extracted: tocainide

KEY WORDS
plasma; derivatization; chiral; acebutolol is IS

REFERENCE
Carr,R.A.; Foster,R.T.; Freitag,D.; Pasutto,F.M. Stereospecific high-performance liquid chromatographic determination of tocainide, *J.Chromatogr.*, **1991**, *566*, 155–162.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 70 mM pH 7 phosphate buffer + 4 mL chloroform:isopentyl alcohol:diethyl ether 71.25:3.75:25, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL chloroform:triethylamine 100:1, add 100 μL 1% (S)-(+)-1-(1-naphthyl)ethyl isocyanate in chloroform, after 1 min add 50 μL 2% ethylchloroformate in chloroform, after 30 s add 50 μL 2.5% ethanolamine in chloroform, inject a 20-125 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μm Nova-Pak silica Radial Pak
Mobile phase: Hexane:chloroform:MeOH 64.5:33:2.5
Flow rate: 2
Injection volume: 20-125

Detector: F ex 245 em 420

CHROMATOGRAM
Retention time: 8.5, 9.5 (enantiomers)
Internal standard: acebutolol

OTHER SUBSTANCES
Extracted: lomefloxacin (F ex 280 em 470)

KEY WORDS
plasma; derivatization; chiral; normal phase; acebutolol is IS

REFERENCE
Foster,R.T.; Carr,R.A.; Pasutto,F.M.; Longstreth,J.A. Stereospecific high-performance liquid chromatographic assay of lomefloxacin in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1243–1248.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 mM pH 7.4 phosphate buffer + 500 μL 2% zinc sulfate in MeOH:water 50:50, mix, centrifuge at 13000 rpm for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 SynChropak bulk support (Knauer)
Column: 120 × 4.6 5 μm Spherisorb ODS1 C18
Mobile phase: MeCN:MeOH:pH 4.5 acetate buffer (ratio not given)
Flow rate: 1
Detector: UV 233

CHROMATOGRAM
Retention time: 4.68

OTHER SUBSTANCES
Extracted: cyclopropane carboxylic acid ester prodrug

KEY WORDS
plasma

REFERENCE
Hovgaard,L.; Brondsted,H.; Buur,A.; Bundgaard,H. Drug delivery studies in Caco-2 monolayers. Synthesis, hydrolysis, and transport of O-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents, *Pharm.Res.*, **1995**, *12*, 387–392.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 235

CHROMATOGRAM

Retention time: 3.80
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μL 50 μg/mL pindolol in MeOH + 150 μL 1 M NaOH + 5 mL diethyl ether, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, add 200 μL 0.1%

S-(+)-1-(1-naphthyl)ethylisocyanate in chloroform, mix for 30 s , inject a 15-200 μL aliquot. Urine. Dilute 100 fold with water, proceed as above.

HPLC VARIABLES

Column: 250 mm long 5 μm Partisil silica
Mobile phase: Hexane:chloroform:MeOH 63:35:2
Flow rate: 2
Injection volume: 15-200
Detector: F ex 220 em 389

CHROMATOGRAM

Retention time: 12 (R), 13 (S)
Internal standard: pindolol (6,7)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Also analyzed: atenolol, nadolol, propranolol, sotalol, toliprolol, tocainide

KEY WORDS

plasma; chiral; derivatization; normal phase

REFERENCE

Piquette-Miller,M.; Foster,R.T.; Pasutto,F.M.; Jamali,F. Stereospecific high-performance liquid chromatographic assay of acebutolol in human plasma and urine, *J.Chromatogr.*, **1990**, *526*, 129–137.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with MeOH and water. Hydrolyze 900 μL serum with β-glucuronidase (EC 3.2.1.31 type H-1 from Helix pomatia) at 60° for 1 h, add 500 μL (?) MeOH, centrifuge at 2000 g, add the supernatant to the SPE cartridge, wash with 1 mL water, dry under vacuum, elute with 2 mL MeOH:water 90:10, filter, inject an aliquot. Urine. 900 μL Urine + 500 μL MeOH, filter, inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm HP C18
Column: 150 × 4.6 5 μm C8P-50 (Asahipak)
Mobile phase: Gradient. MeOH:buffer 30:70 for 4 min, to 45:55 over 6 min, to 50:50 over 2 min, to 60:40 over 2 min, re-equilibrate at initial conditions for 10 min. (Prepare buffer by mixing 100 mM NaH_2PO_4 and 100 mM Na_2HPO_4 to achieve a pH of 7.0 and adding 10 mM N-cetyl-N,N,N-trimethylammonium bromide.)
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Extracted: alprenolol, atenolol, metoprolol, oxprenolol, propranolol

KEY WORDS

serum; comparison with CE; SPE

REFERENCE

Lukkari,P.; Sirén,H. Ion-pair chromatography and micellar electrokinetic capillary chromatography in analyzing β-adrenergic blocking agents from human biological fluids, *J.Chromatogr.A*, **1995**, *717*, 211–217.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 233.4

CHROMATOGRAM

Retention time: 10.233

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrospher 60 RP Select B

Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.10, k' 4.20 (enantiomers)

OTHER SUBSTANCES
Also analyzed: alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: formulations
Sample preparation: Weigh 10 tablets, powder finely. Weigh accurately powder containing 10 mg nifedipine, dissolve in MeOH and make up to 50 mL with MeOH. Add 1.6 mg IS, filter through a 45 μm membrane filter. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP C18
Mobile phase: MeOH:water 55:45 pH 4.5
Flow rate: 1.0 for 4 min, then 2.0
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 3.31
Internal standard: oxprenolol (4.35)
Limit of detection: 3.01 μg/mL

OTHER SUBSTANCES
Simultaneous: nifedipine, nifedipine oxidation products

KEY WORDS
comparison with GC and first-derivative spectroscopy; tablets

REFERENCE
el Walily,A.F.M. Analysis of nifedipine--acebutolol hydrochloride binary combination in tablets using UV-derivative spectroscopy, capillary gas chromatography and high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 21–30.

SAMPLE
Matrix: formulations
Sample preparation: Take up in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb C2

Mobile phase: MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: atenolol, nadolol, alprenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol
Interfering: timolol

KEY WORDS
tablets

REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL of an aqueous solution with 1 mL 100 mM nickel sulfate in water, 1 mL 20% aqueous ammonia, and 5 mL chloroform:carbon disulfide 98:2, shake vigorously for 1 min, wash the organic layer with three 2 mL portions of water, filter (phase-separation paper). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot. (Copper may also be used with electrochemical detection or UV detection at 270 nm.)

HPLC VARIABLES
Guard column: 30 × 4 40 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeOH:20 mM pH 5.8 sodium acetate buffer 80:20 containing 5 mM lithium perchlorate
Flow rate: 1.5
Injection volume: 10
Detector: UV 325, E, Merck-Clevenot E 230, Model LCC 231 thin-layer electrolytic cell with a glassy carbon electrode at +0.7 V, standard calomel reference electrode

CHROMATOGRAM
Retention time: k' 2.48
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES
Also analyzed: alprenolol, ephedrine, flecainide, methamphetamine, propranolol

KEY WORDS
derivatization; complexation

REFERENCE
Leroy,P.; Nicolas,A. Determination of secondary amino drugs as their metal dithiocarbamate complexes by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *317*, 513–521.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.2

OTHER SUBSTANCES

Also analyzed: acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 internal surface reversed-phase silica (Pinkerton) (Regis Chemical)
Mobile phase: Isopropanol:100 mM pH 6.8 KH_2PO_4 10:90
Flow rate: 1
Injection volume: 10
Detector: UV 232-274 (wavelength of maximum absorption used)

CHROMATOGRAM
Retention time: 32.2

OTHER SUBSTANCES
Simultaneous: carteolol, atenolol, metoprolol, oxprenolol, pindolol, alprenolol

REFERENCE
Ohshima,T.; Takagi,K.; Miyamoto,K.-I. High performance liquid chromatographic retention time of β-blockers as an index of pharmacological activity, *J.Liq.Chromatogr.*, **1993**, *16*, 3933–3939.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 30:70 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 2.86 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 2.54

OTHER SUBSTANCES
Also analyzed: bunitrolol, carazolol, celiprolol, esmolol, mepindolol, metoprolol, timolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol:diethylamine 90:10:0.1
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 25.7

KEY WORDS
narrow-bore; chiral; α 1.12

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 244

KEY WORDS
chiral; α = 1.09 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:EtOH:diethylamine 90:10:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 320

CHROMATOGRAM
Retention time: k' 2.77, 3.19 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.00

OTHER SUBSTANCES
Also analyzed: alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.51 (A), 3.72 (B)

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine,

pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL 55 mM N-Benzyloxycarbonyl-L-phenylalanine (N-CBZ-L-Phe) in dichloromethane + 100 μL 14 mM N,N-dimethylaminopyrine (dimethylaminopyridine (?)) in dichloromethane + 100 μL 9-acetylanthracene in dichloromethane, cool in an ice bath, add 500 μL of a solution of acebutolol in dichloromethane, add 100 μL 240 mM dicyclohexylcarbodiimide in dichloromethane, shake mechanically at 0° for 30 min, add 100 μL 1.06 M acetic anhydride in dichloromethane, shake mechanically at 30° for 15 min, add 1 mL MeOH, mix, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Nova-Pak C18 precolumn
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:water 60:40
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM
Retention time: 14.13 (S), 15.74 (R)
Internal standard: 9-acetylanthracene (6.52)

KEY WORDS
derivatization; chiral

REFERENCE
Wen,Y.H.; Wu,S.S.; Wu,H.L. Chiral separation of acebutolol by derivatization and high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 3329–3345.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of a 1 mM solution in MeOH or water with 50 μL pH 8 borate buffer and 50 μL 18 mM 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate in acetone, vortex, let stand at room temperature for 30 min, add 100 μL 10 mM trans-4-hydroxy-L-proline in water, mix, let stand for 2 min, add 2 mL dichloromethane, vortex for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an aliquot. Prepare 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate as follows. Stir 1.5 mmoles lithium aluminum hydride in THF, slowly add 2 mmoles (S)-naproxen in 20 mL anhydrous THF, reflux for 1 h, evaporate most of the solvent, cautiously add water with stirring, acidify with 6 N HCl,

extract three times with diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane:MeOH 100:2 (flash chromatography), evaporate eluate to dryness, dry under vacuum over KOH to give 2-(6-methoxy-2-naphthyl)propanol as a white solid (mp 92-3°). Stir 0.5 mmoles 2-(6-methoxy-2-naphthyl)propanol and 0.5 mmoles triethylamine in 10 mL dry toluene at 0°, add 1 mL 20% phosgene in toluene (Caution! Phosgene is highly toxic, perform reaction in a chemical fume hood!) (Fluka), stir for 4 h, filter, evaporate to dryness under reduced pressure, dry under vacuum to give 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (mp 60°). Store under vacuum over phosphorus pentoxide at room temperature.)

HPLC VARIABLES

Column: 250 × 4 5 μm Zorbax-SIL
Mobile phase: n-Hexane:isopropanol 100:5
Flow rate: 1.5
Injection volume: 100
Detector: UV 230, F ex 270 em 365

CHROMATOGRAM

Retention time: k' 20.4, 21.9 (enantiomers)

KEY WORDS

derivatization; chiral; normal phase

REFERENCE

Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

SAMPLE

Matrix: urine
Sample preparation: Direct injection.

HPLC VARIABLES

Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. MeCN:100 mM sodium carbonate 4.5:95.5, after 0.1 min MeCN: water 4.5:95.5, inject, stay with this mobile phase for 5 min then go to MeCN:water 67.5: 32.5 over 15 min, re-equilibrate for 10 min before next injection.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 12

KEY WORDS

rat

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: urine
Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile

phase B, monitor the effluent from column B. For gradient elution, after 15 min re-equilibrate both columns for 12.5 min before the next injection. For isocratic elution, remove column A from the circuit after 1.25 min, re-equilibrate column A for 1.5 min. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (for screening) or isocratic 22:78 (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 235

CHROMATOGRAM

Retention time: 11.5 (gradient), 6 (isocratic)
Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: (using gradient elution) metabolites, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol
Interfering: timolol

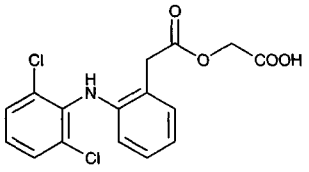
KEY WORDS

column-switching

REFERENCE

Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

Aceclofenac



Molecular formula: $C_{16}H_{13}Cl_2NO_4$
Molecular weight: 354.19
CAS Registry No.: 89796-99-6
Merck Index: 19

SAMPLE
Matrix: blood
Sample preparation: Acidify plasma, extract with hexane:isopropanol 90:10, evaporate, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Spherisorb 5C8
Mobile phase: MeOH:70 mM pH 5.8 phosphate buffer 49:51
Flow rate: 1.7
Detector: UV 282

CHROMATOGRAM
Internal standard: flufenamic acid, niflumic acid
Limit of detection: 3 pmole

OTHER SUBSTANCES
Extracted: metabolites, diclofenac

KEY WORDS
plasma; rat; human; monkey; pharmacokinetics

REFERENCE
Bort,R.; Ponsoda,X.; Carrasco,E.; Gómez-Lechón,M.J.; Castell,J.V. Comparative metabolism of the non-steroidal antiinflammatory drug, aceclofenac, in the rat, monkey, and human, *Drug Metab.Dispos.*, **1996**, *24*, 969–975.

SAMPLE
Matrix: blood, microsomal incubations, urine
Sample preparation: Deconjugate plasma and urine samples with 50 mU/mL β-glucuronidase and 30 mU/mL arylsulfatase in 100 mM pH 4.5 acetate buffer containing 120 mM NaF, heat at 37° for 4 h. Add an equal volume of MeCN, mix, centrifuge at 9000 rpm for 10 min. Add an equal volume of 100 mM pH 7.4 phosphate buffer to the supernatant, mix, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5μm Spherisorb ODS2
Mobile phase: MeCN: 0.02% triethanolamine in 100 mM pH 7.4 phosphate buffer 25:75
Flow rate: 1
Detector: UV 282

CHROMATOGRAM
Retention time: 23
Internal standard: carprofen (11)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; urine; metabolites

REFERENCE
Bort,R.; Ponsoda,X.; Carrasco,E.; Gómez-Lechón,M.J.; Castell,J.V. Metabolism of aceclofenac in humans, *Drug Metab.Dispos.*, **1996**, *24*, 834–841.

SAMPLE
Matrix: cultured hepatocytes, microsomal incubations
Sample preparation: Cultured hepatocytes. Dilute the incubation mixture with an equal volume of MeCN, centrifuge at 9000 rpm for 20 min, dilute 50:50 with 100 mM pH 7.4 phosphate buffer, inject an aliquot. Microsomal incubations. Add an equal volume of cold MeCN to the microsomal incubation, centrifuge, dilute 50:50 with 100 mM pH 7.4 phosphate buffer, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Spherisorb ODS2
Mobile phase: MeCN:100 mM phosphate buffer containing 0.02% triethanolamine 25:75
Flow rate: 1
Detector: UV 282

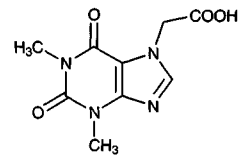
CHROMATOGRAM
Retention time: 23
Internal standard: carprofen (11)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; human; pharmacokinetics

REFERENCE
Bort,R.; Ponsoda,X.; Carrasco,E.; Gómez-Lechón,M.J.; Castell,J.V. Comparative metabolism of the non-steroidal antiinflammatory drug, aceclofenac, in the rat, monkey, and human, *Drug Metab.Dispos.*, **1996**, *24*, 969–975.

Acefylline



Molecular formula: $C_9H_{10}N_4O_4$
Molecular weight: 238.20
CAS Registry No.: 652-37-9, 837-27-4 (sodium salt)
Merck Index: 22

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 3.628

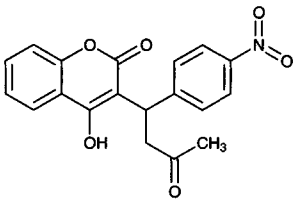
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acenocoumarol



Molecular formula: $C_{19}H_{15}NO_6$
Molecular weight: 353.33
CAS Registry No.: 152-72-7
Merck Index: 29

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 283

CHROMATOGRAM

Retention time: 4.91

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 204

CHROMATOGRAM

Retention time: 20.052

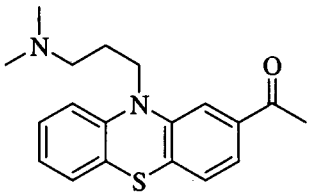
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acepromazine



Molecular formula: $C_{19}H_{22}N_2OS$
Molecular weight: 326.46
CAS Registry No.: 61-00-7, 3598-37-6 (maleate)
Merck Index: 32

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 243

CHROMATOGRAM

Retention time: 6.77
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 249.9

CHROMATOGRAM

Retention time: 10.763

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 12.81 (A), 5.81 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES

Guard column: 10 × 2.1 37-50 μm Bondapak C18

Column: 300 × 3.9 Bondapak C18

Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid

Flow rate: 1.2

Injection volume: 50

Detector: UV 240

CHROMATOGRAM

Retention time: 12

Limit of detection: 2 ng/g

OTHER SUBSTANCES

Extracted: azaperol, carazolol, xylazine, azaperone, haloperidol, propiomazine, chlorpromazine

KEY WORDS

SPE; pig; kidney

REFERENCE

Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES

Guard column: Hypersil 5 μm SAS C1

Column: 250 mm long 5 μm Hypersil SAS C1

Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate

Flow rate: 2

Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM

Retention time: 18
Limit of detection: 2 ng/g

OTHER SUBSTANCES

Extracted: azaperol, azaperone, carazolol, xylazine, haloperidol, propriomazine, chlorpromazine

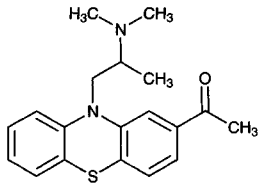
KEY WORDS

SPE; pig; kidney; liver

REFERENCE

Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

Aceprometazine



Molecular formula: $C_{19}H_{22}N_2OS$
Molecular weight: 326.46
CAS Registry No.: 13461-01-3

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 242

CHROMATOGRAM

Retention time: 6.20

Limit of detection: <120 ng/mL

KEY WORDS

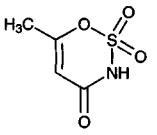
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; tri-

fluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Acesulfame



Molecular formula: $C_4H_5NO_4S$
Molecular weight: 163.15
CAS Registry No.: 33665-90-6

SAMPLE

Matrix: beverages, sweetener
Sample preparation: Sweetener. Dissolve 30 mg powdered tabletop sweetener in water and dilute to 25 mL, filter (0.2 μm PTFE). Beverages. Dilute fruit juice 1:10 with water. Degas carbonated beverages in a ultrasonic bath for 5 min, dilute 1:10 with water, filter. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4 Dionex IonPak AG4A-SC
Column: 250 × 4 Dionex IonPak AS4A-SC
Mobile phase: Gradient. A was 1 mM sodium carbonate. B was 12.5 mM sodium carbonate. A:B 100:0 for 4.5 min, from 100:0 to 0:100 over 1 min, maintain at 0:100 for 22.5 min, from 0:100 to 100:0 over 0.1 min
Flow rate: 1
Injection volume: 50
Detector: UV 190 for 6 min, UV 206 22 min, then UV 190; Conductivity, Dionex ED40 in conductivity mode preceded by a Dionex ASRS-I suppressor (external water mode, 300 mA)

CHROMATOGRAM

Retention time: 14
Limit of detection: 44 ng/mL (UV), 230 ng/mL (conductivity)

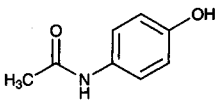
OTHER SUBSTANCES

Simultaneous: aspartame, saccharin

REFERENCE

Chen,Q.-C.; Mou,S.-; Liu,K.-; Yang,Z.-; Ni,Z.-. Separation and determination of four artificial sweeteners and citric acid by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *771*, 135–143.

Acetaminophen



Molecular formula: $C_8H_9NO_2$
Molecular weight: 151.16
CAS Registry No.: 103-90-2
Merck Index: 45

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 10 μL 200 μg/mL IS and 1 mL pH 7.4 phosphate buffer, vortex briefly, add 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 1 mL MeOH and inject a 20 μL aliquot of the solution.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II ODS
Mobile phase: MeCN:acetic acid:water 10:5:85
Flow rate: 1
Injection volume: 20
Detector: UV 255

CHROMATOGRAM

Retention time: 3.3
Internal standard: 2-acetoamidophenol (6.4)
Limit of quantitation: 2 μg/mL

KEY WORDS

liver; lung; muscle; urine; pericardial fluid

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 1.0 M perchloric acid, centrifuge. Add 200 μL 700 mM potassium phosphate, cool on ice for 30 min, centrifuge for 5 min. Inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 C8 Rainin "Short One"
Mobile phase: MeCN:buffer 4:96 (Buffer was 25 mM phosphate with 0.5% acetic acid, pH 3.1)
Flow rate: 1.0
Detector: E, HP 1049A amperometric detector, 700 mV, oxidation mode

CHROMATOGRAM

Retention time: 6.5

KEY WORDS
plasma

REFERENCE
Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE
Matrix: blood
Sample preparation: Inject a 5-20 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 5:95 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B)
Flow rate: 1
Injection volume: 5 (A), 20 (B)
Detector: UV 254

CHROMATOGRAM
Retention time: 4.99 (A), 6 (B)

OTHER SUBSTANCES
Extracted: barbital (B), carbamazepine (B), phenobarbital (B), phenytoin (B), primidone (B), sulfapyridine (B), theophylline (A),
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 5.592

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Add 50 mL of mobile phase to 0.5 g of sample and swirl to aid dissolution. Dilute to 100 mL with mobile phase. Dilute 1:10, filter (0.22 μm nylon). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:water:triethylamine:acetic acid 5.5:94.1:0.2:0.2
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 0.7

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Weigh 500 mg homogenized analgesic powder, transfer to 100 mL volumetric flask, add ca. 50 mL mobile phase, swirl and dilute to volume with mobile phase. Dilute an aliquot of this solution 1:10 with mobile phase, filter (0.20 μm Cameo nylon filter, MSI, Westboro, MA) an aliquot, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:triethylamine:acetic acid:water 5.5:0.2:0.2:94.1 (Prepare mobile phase as follows. Mix 110 mL MeCN, 4 mL triethylamine, 4 mL glacial acetic acid and make up to 2 L with water.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 0.7

OTHER SUBSTANCES
Extracted: aspirin, caffeine
Noninterfering: salicylic acid

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out powdered sample containing 51 mg acetaminophen, add 80 mL MeOH, sonicate for 10 min, dilute to 100 mL with MeOH, centrifuge. Remove a 5 mL aliquot of the supernatant and add it to 1 mL 2 mg/mL resorcinol, add 2 mL MeOH, make up to 20 mL with 50 mM pH 3.0 triethylamine phosphate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: THF:50 mM pH 3.0 triethylamine phosphate 12:88
Flow rate: 0.6
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 15
Internal standard: resorcinol (9)

OTHER SUBSTANCES
Simultaneous: aspirin (post-column irradiation gives an increase in peak height), caffeine, propyphenazone

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S10-ODS2
Mobile phase: MeOH:20 mM pH 4.0 acetate buffer 13:87
Flow rate: 1.3
Detector: UV 243, UV 254

CHROMATOGRAM
Internal standard: 2-acetamidophenol

REFERENCE
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE

Matrix: urine

Sample preparation: Unhydrolyzed urine. 200 μL Urine + 600 μL 200 mM pH 5.0 sodium acetate/acetic acid buffer, filter through a 10 kDa molecular weight cut-off membrane (Alltech) while centrifuging. Inject a 5 μL aliquot of the filtrate. Hydrolyzed urine. 500 μL Urine + 500 μL 125 mM pH 5.0 sodium acetate buffer containing 40 μL β-glucuronidase/sulfatase, incubate overnight at 37°. Filter a 500 μL aliquot through a 10 kDa molecular weight cut-off membrane (Alltech) while centrifuging. Inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 100 mm long 3 μm Microsorb-MV C8

Mobile phase: Gradient. MeCN:MeOH:buffer from 0:5:95 to 0:9:91 over 10 min, to 0:20:80 over 10 min, to 5:20:75 over 1 min, to 30:20:50 over 1 min. (Buffer was 25 mM pH 3.4 dibasic ammonium phosphate-acetate, 0.15% (sic).)

Injection volume: 5-10

Detector: UV 254

CHROMATOGRAM

Retention time: 16-17

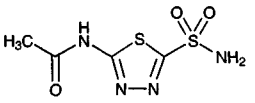
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

Acetazolamide



Molecular formula: $C_4H_6N_4O_3S_2$
Molecular weight: 222.25
CAS Registry No.: 59-66-5, 1424-27-7 (sodium salt)
Merck Index: 50
Lednicer No.: 1 249, 1 111

SAMPLE

Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 5.8
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: amitriptyline, norfluoxetine, trimipramine

KEY WORDS

plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 4.89
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES
Extracted: bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim
Interfering: ampicillin

KEY WORDS
serum; plasma; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, urine
Sample preparation: To 500 μL whole blood, plasma, or urine add 2.5 mL 50% ammonium sulfamate, vortex for 30 s. (Place the tube containing whole blood in boiling water for 30 s and then quickly in cold water.) Add 5 mL ethyl acetate, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 5 mL pH 8.0 phosphate buffer, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 500 μL pH 10.0 glycine buffer, vortex for 30 s, centrifuge at 3000 g for 5 min. Aspirate and discard the organic layer, add 500 μL ether to the remaining glycine buffer layer, vortex for 1 min, discard the ether phase. Vent the aqueous layer for about 30 min and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Altima C18 (Alltech)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate 20:80
Flow rate: 1
Injection volume: 20
Detector: 285

CHROMATOGRAM
Retention time: 4.55
Internal standard: acetazolamide

KEY WORDS
plasma; whole blood; acetazolamide is IS

REFERENCE
Iyer,G.R.; Taft,D.R. Determination of methazolamide concentrations in human biological fluids using high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1021–1027.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 265.3

CHROMATOGRAM
Retention time: 6.927

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: perfusate, urine
Sample preparation: Mix 350 μL perfusate or urine with 350 μL MeCN. Add 26 μL 1 mg/mL IS, vortex, centrifuge at 10000 g for 10 min, add 1 mL dichloromethane to 500 μL of the resultant supernatant, vortex, centrifuge at 10000 g for 20 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: Resolvex C18 (Fisher Scientific, Pittsburgh, PA)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate buffer 15:85
Flow rate: 1

Injection volume: 50

CHROMATOGRAM
Internal standard: sulfadiazine

KEY WORDS
rat; kidney

REFERENCE
Taft,D.R.; Chapron,D.J.; Fournier,D.J.; Sweeney,K.R. Concentration-dependent tubular secretion of acetazolamide and its inhibition by salicylic acid in the isolated perfused rat kidney, *Drug Metab.Dispos.*, **1996**, *24*, 456–461.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak NH2
Mobile phase: MeOH:10 mM ammonium formate 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Limit of detection: 20 ng

KEY WORDS
rabbit; buffer

REFERENCE
Tang-Liu,D.D.-S.; Richman,J.B.; Weinkam,R.J.; Takruri,H. Effects of four penetration enhancers on corneal permeability of drugs in vitro, *J.Pharm.Sci.*, **1994**, *83*, 85–90.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 4.77 (A), 3.68 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem,

diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 µL 50 µg/mL β-hydroxyethyltheophylline in MeOH, inject 5 µL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 4.30 (A); B (5.2)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, flumethiazide, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 0.3:0.7:99:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 3.9

Limit of detection: <10 ng (by MS)

OTHER SUBSTANCES

Extracted: hydrochlorothiazide

REFERENCE

Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g NaH_2PO_4:Na_2HPO_4 99:1 + 0.5 g NaCl (final pH 5-5.5), add 4 mL ethyl acetate, extract in a mechanical agitator for 10 min. Remove the organic layer and add it to 2 mL 5% lead acetate, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, filter (15 mm nylon Teknokroma 0.45 μm filter), inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 45 μm HP-Hypersil ODS-C18

Mobile phase: Gradient. MeCN:buffer from 12:88 to 15:85 over 3 min, to 40:60 after another 2 min, maintain at 40:60 (Buffer was 3.45 g $NaH_2PO_4.H_2O$ in 500 mL water and 0.7 mL propylamine hydrochloride, pH adjusted to 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 275

CHROMATOGRAM
Retention time: 5.4
Internal standard: β-hydroxyethyltheophylline (4.6)
Limit of detection: 8 ng/mL

OTHER SUBSTANCES
Simultaneous: theobromine, theophylline, 1,7-dimethylxanthine, caffeine

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of acetazolamide in human urine samples by reversed-phase high-performance liquid chromatography in the presence of xanthines, *J.Chromatogr.*, **1992**, *582*, 181–187.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 270

CHROMATOGRAM
Retention time: 9.8
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 2.2
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 2000 ng/mL

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, amiloride, bendroflumethiazide, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine
Sample preparation: Inject a 5 μL aliquot directly onto column A with mobile phase A and elute to waste, after 0.3 min elute from column A onto column B with mobile phase A for 1.2 min then remove column A from the circuit and elute column B with mobile phase B, starting the gradient. Three minutes after the start change mobile phase A to MeCN:mobile phase A buffer 50:50 over 2 min to clean column A.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS C18; B 125 × 4 5 μm HP-LiChrospher 100 RP 18
Mobile phase: A 3.45 g $NaH_2PO_4.H_2O$ in 500 mL water, pH adjusted to 3 with concentrated phosphoric acid; B Gradient. MeCN:water 0:100 for 2 min then to 50:50 over 2 min, maintain at 50:50
Injection volume: 5
Detector: UV 275

CHROMATOGRAM

Retention time: 5.27
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: caffeine, theophylline, theobromine, 1,7-dimethylxanthine

KEY WORDS

column-switching; heart-cut

REFERENCE

Campíns-Falcó,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Application of column-switching techniques to the determination of medium polarity drugs: determination of acetazolamide in urine, *J.Chromatogr.B*, **1994**, *654*, 85–90.

SAMPLE

Matrix: urine

Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18

Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 5.4

Limit of detection: 40 ng/mL.

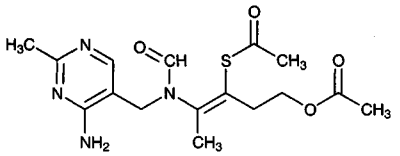
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Acetiamine



Molecular formula: $C_{16}H_{22}N_4O_4S$
Molecular weight: 366.44
CAS Registry No.: 299-89-8, 28008-04-0 (HCl)
Merck Index: 51

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.78

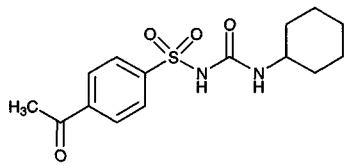
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acetohexamide



Molecular formula: $C_{15}H_{20}N_2O_4S$
Molecular weight: 324.40
CAS Registry No.: 968-81-0
Merck Index: 59
Lednicer No.: 1 138

SAMPLE

Matrix: blood, urine
Sample preparation: Dilute urine 5 times with water. 200 μL Plasma or diluted urine + 2 mL 100 mM pH 5.0 phosphate buffer + 5 mL cyclohexamide in benzene:ethyl acetate 1:1 (Caution! Benzene is a carcinogen!), shake, centrifuge at 3000 rpm for 20 min. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5 C18
Mobile phase: MeCN:0.2% acetic acid 50:50
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Internal standard: cyclohexamide

OTHER SUBSTANCES

Extracted: metabolites, hydroxyhexamide

KEY WORDS

plasma; rat; guinea pig; rabbit

REFERENCE

Asada,S.; Nagamine,S.; Nakae,H. Comparative pharmacokinetics of acetohexamide and its metabolite, hydroxyhexamide in laboratory animals, *Chem.Pharm.Bull.(Tokyo)*, **1989**, *37*, 760–765.

SAMPLE

Matrix: erythrocytes
Sample preparation: 1 mL erythrocyte mixture + 100 μL 10 μM tolazamide in MeOH, extract with toluene:ethyl acetate 1:1. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:0.2% acetic acid 47:53
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Internal standard: tolazamide

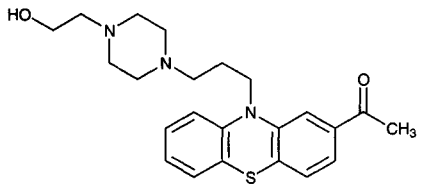
OTHER SUBSTANCES

Extracted: hydroxyhexamide

REFERENCE

Kishimoto,M.; Kawamori,R.; Kamada,T.; Inaba,T. Carbonyl reductase activity for acetohexamide in human erythrocytes, *Drug Metab.Dispos.*, **1994**, *22*, 367–370.

Acetophenazine



Molecular formula: $C_{23}H_{29}N_3O_2S$
Molecular weight: 411.57
CAS Registry No.: 2751-68-0, 5714-00-1 (dimaleate)
Merck Index: 70

SAMPLE

Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7.4
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, trifluopromazine, trimeprazine
Interfering: trihexyphenidyl

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: mesoridazine, promethazine, chlorpromazine, thioridazine, prochlorperazine, butaperazine, thiethylperazine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.33

OTHER SUBSTANCES
Simultaneous: trifluprom azine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, thiothixene, reserpine, ethopropazine, promethazine, propiomazine
Interfering: deserpidine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bi-

bucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 9.83 (A), 4.89 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

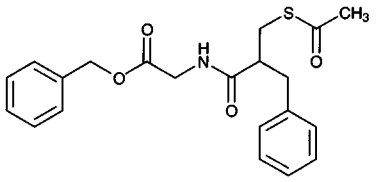
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Acetorphan



Molecular formula: $C_{21}H_{23}NO_4S$
Molecular weight: 385.48
CAS Registry No.: 81110-73-8
Merck Index: 72

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 22.495

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize kidney in 30 volumes of 50 mM pH 7.4 Tris-HCl buffer (Ultra-Turrax homogenizer), filter through a GF/B filter presoaked in 0.3% polyethylenimine, wash filter three times with 4 mL 50 mM pH 7.4 Tris-HCl buffer. Extract filter with 1 mL EtOH, centrifuge extract, inject an aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:100 mM pH 6.2 ammonium acetate 70:30
Flow rate: 1
Detector: UV 210

OTHER SUBSTANCES

Extracted: metabolites, thiorphan

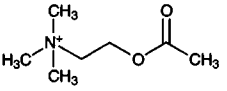
KEY WORDS

mouse; kidney

REFERENCE

De la Baume,S.; Brion,F.; Dam Trung Tuong,M.; Schwartz,J.C. Evaluation of enkephalinase inhibition in the living mouse, using [^{3}H]acetorphan as a probe, *J.Pharmacol.Exp.Ther.*, **1988**, *247*, 653–660.

Acetylcholine



Molecular formula: $C_7H_{16}ClNO_2$ (chloride)
Molecular weight: 181.66 (chloride)
CAS Registry No.: 51-84-3, 60-31-1 (chloride), 66-23-9 (bromide)
Merck Index: 87

SAMPLE

Matrix: CSF, dialysate, tissue
Sample preparation: Dialysate. Inject dialysate directly. Tissue. Homogenize brain tissue with 10 volumes 100 mM perchloric acid (Potter-Elvehjem), let stand on ice for 15 min, centrifuge at 4000 g for 15 min, inject a 0.5 μL aliquot. CSF. Deproteinize by passing through a 0.02 μm Anatop 10 syringe filter (Alltech), inject a 0.5 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 1 PEEK column packed with Aminex A-9 (Bio-Rad)
Mobile phase: 200 mM pH 8.0 K/Na 3/1 phosphate buffer containing 5 mM NaCl and 0.1% Kathon CG
Column temperature: 25
Flow rate: 0.06 (obtained with a flow splitter)
Injection volume: 0.5
Detector: E, AMOR (Spark Holland), platinum working electrode + 250 mV, carbon composite auxiliary electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent flowed through a reactor which had 4 U acetylcholine esterase (EC 3.1.1.7 type VI-S from electric eel, 260 IU/mg) and 4 U choline oxidase (EC 1.1.3.17 from Alcaligenes sp., 12.7 IU/mg) enclosed between two 0.01 μm cellulose nitrate filters (Sartorius) (construction details given) to the detector.

CHROMATOGRAM

Retention time: 12
Limit of detection: 10 fmole

OTHER SUBSTANCES

Extracted: choline

KEY WORDS

use metal-free tubing and connectors; solvent reservoir; column; reactor; and detector maintained at 25; rat; brain; human; post-column reaction; immobilized enzyme reactor

REFERENCE

Flentge,F.; Venema,K.; Koch,T.; Korf,J. An enzyme-reactor for electrochemical monitoring of choline and acetylcholine: applications in high-performance liquid chromatography, brain tissue, microdialysis and cerebrospinal fluid, *Anal.Biochem.*, **1992**, *204*, 305–310.

SAMPLE

Matrix: blood
Sample preparation: Add neostigmine and ethylhomocholine to plasma or red blood cells. 150 μL Plasma or red blood cells + 1 mL 400 mM perchloric acid, let stand at 0-4° for 30 min, centrifuge at 5500 g for 1 min. Remove a 750 μL aliquot of the supernatant and add it to 36 μL 10 M potassium acetate, let stand at 0-4° for 5 min, centrifuge at 5500 g for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 1.9 Chrompack reversed phase

Column: 100 × 3 Chromspher 5 C18 (Chrompack) (Prepare column by washing with MeOH, MeOH:water 50:50, water, and 5 mg/mL sodium laurylsulfate in water (each wash 20 min at 1 mL/min). Thoroughly wash pump with water (column off line), wash column with water for 5 min and mobile phase for 1 h. Column should be disconnected from the pre-column, reactor, and detector. [Chromatographia, 1987,24,827].)
Mobile phase: 200 mM pH 8.0 potassium phosphate buffer containing 5 mM KCl
Flow rate: 0.6
Injection volume: 100
Detector: E, Spark Holland Amor, Pt working electrode +500 mV, carbon composite auxiliary electrode, Ag/AgCl reference electrode following an enzyme reactor. (Reactor was a 10 × 2.1 Hypersil APS-2 column, activate with glutaraldehyde, equilibrate with mobile phase for 20 min. Inject 80 U acetylcholine esterase (EC 3.1.1.7, type VI-S from electric eel, 260 IU/mg) and 40 U choline oxidase (EC 1.1.3.17 from Alcaligenes sp., 12.7 IU/mg) in 500 μL mobile phase onto the reactor and pump through at 0.05 mL/min for 20 min with mobile phase. [Chromatographia, 1987,24,827])

CHROMATOGRAM
Retention time: 9
Internal standard: ethylhomocholine (5)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
plasma; red blood cells; human; mouse

REFERENCE
Damsma,G.; Flentge,F. Liquid chromatography with electrochemical detection for the determination of choline and acetylcholine in plasma and red blood cells. Failure to detect acetylcholine in blood of humans and mice, *J.Chromatogr.*, **1988**, *428*, 1–8.

SAMPLE
Matrix: dialysate
Sample preparation: Inject 10 μL of rat brain dialysate.

HPLC VARIABLES
Guard column: ACH-3-G guard cartridge (ESA)
Column: 150 × 3 5 μm ACH-3 polymeric reversed-phase column (ESA)
Mobile phase: 100 mM sodium phosphate + 0.5 mM tetramethylammonium chloride + 0.005% Reagent MB (a microbicide from ESA) + 2 mM octanesulfonic acid, final pH 8.0
Column temperature: 35
Flow rate: 0.35
Injection volume: 10
Detector: E, ESA Coulochem Model 5200A, Model 5040 analytical cell, palladium reference electrode, stainless steel counter electrode, platinum working electrode + 300 mV following a solid-phase reactor containing immobilized acetylcholinesterase and choline oxidase (reactor temp 35)

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 3 μM

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
brain; rat

REFERENCE
Greaney,M.D.; Marshall,D.L.; Bailey,B.A.; Acworth,I.N. Improved method for the routine analysis of acetylcholine release in vivo: quantitation in the presence and absence of esterase inhibitor, *J.Chromatogr.*, **1993**, *622*, 125–135.

SAMPLE
Matrix: dialysate
Sample preparation: Inject 5 μL aliquots of the dialysate (Ringer's solution).

HPLC VARIABLES
Column: 530 × 1 cation exchange MF-8904 (Bioanalytical Systems)
Mobile phase: 50 mM Na_2HPO_4 containing 5 mL/L Kathon CG (Bioanalytical Systems CF-2150) (Mobile phase was only partially degassed; some oxygen is essential for the enzyme reactor.)
Flow rate: 0.14
Injection volume: 5
Detector: E, Bioanalytical Systems LC-4C, peroxidase-redox polymer coated glassy carbon electrode +100 mV (Anal.Chem. 1992, 64, 3084), Ag/AgCl reference electrode. The column effluent passed through a 50 × 1 immobilized-enzyme reactor containing acetylcholinesterase (EC 3.1.1.7) and choline oxidase (EC 1.1.3.17) (Bioanalytical Systems MF-8903) and flowed to the detector.

CHROMATOGRAM
Retention time: 20
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
rat

REFERENCE
Huang,T.; Yang,L.; Gitzen,J.; Kissinger,P.T.; Vreeke,M.; Heller,A. Detection of basal acetylcholine in rat brain microdialysate, *J.Chromatogr.B*, **1995**, *670*, 323–327.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 10 μL aliquot of dialysate onto a 55 × 1 reactor containing immobilized choline oxidase and catalase (BAS). (The enzymes destroy choline but not acetylcholine. Acetylcholine is eluted from the reactor onto the analytical column.)

HPLC VARIABLES
Column: 530 × 1 10 μm ACh (BAS)
Mobile phase: 28 mM pH 8.5 Na_2HPO_4 containing 0.5% of 1% Kathon antimicrobial solution (BAS)
Flow rate: 0.1
Injection volume: 10
Detector: E, Pt electrode +0.5 V, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 50 × 1 reactor packed with immobilized acetylcholine oxidase and choline oxidase (BAS) to the detector. (Acetylcholine was converted to hydrogen peroxide which was then detected.)

CHROMATOGRAM
Retention time: 13
Limit of detection: 5 nM
Limit of quantitation: 10 nM

KEY WORDS
post-column reaction; rat; immobilized enzyme reactor

REFERENCE
Tsai,T.-R.; Cham,T.-M.; Chen,K.-C.; Chen,C.-F.; Tsai,T.-H. Determination of acetylcholine by on-line microdialysis coupled with pre- and post-microbore column enzyme reactors with electrochemical detection, *J.Chromatogr.B*, **1996**, *678*, 151–155.

SAMPLE
Matrix: formulations
Sample preparation: Make up the lyophilized preparation in sterile water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Mix (? g) sodium 1-heptanesulfonate (Waters PIC Reagent B-7) in 900 mL water, adjust pH to 4.0 with 6 M ammonium hydroxide, add 50 mL MeCN, make up to 1 L with water
Flow rate: 2
Injection volume: 50
Detector: RI

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: choline
Noninterfering: acetic acid, mannitol

KEY WORDS
stability-indicating

REFERENCE
Tao,F.T.; Thurber,J.S.; Dye,D.M. High-performance liquid chromatographic determination of acetylcholine in a pharmaceutical preparation, *J.Pharm.Sci.*, **1984**, *73*, 1311–1313.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in saline, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 20 mL Low-UV PIC B-7 (Waters) diluted with 480 mL water (10 mM 1-heptanesulfonic acid).)
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 12.6

REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

SAMPLE
Matrix: solutions

Sample preparation: solutions

HPLC VARIABLES

Column: 30 × 2.1 Aquapore AX300 (Brownlee) (Prepare the column by injecting ten 20 μL aliquots of 10 mg/mL choline oxidase (from Alcaligenes sp, 33 U/mg) and 100 μL cholinesterase (Type III, from electric eel, 970 U/mg, 0.65 mg/mL). Replenish enzymes every 1-2 weeks. Acetylcholine is converted to choline and choline is converted to hydrogen peroxide.)
Mobile phase: 20 mM pH 7 Tris-acetate buffer containing 1 mM tetramethylammonium chloride and 200 μM octanesulfonate
Flow rate: 2
Detector: E, BAS LC4B, Pt working electrode +0.5 V, Ag/AgCl reference electrode

KEY WORDS

post-column reaction; immobilized enzyme reactor

REFERENCE

Meek,J.L.; Eva,C. Enzymes adsorbed on an ion exchanger as a post-column reactor: application to acetylcholine measurement, *J.Chromatogr.*, **1984**, *317*, 343–347.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 4 mM solution in water, inject a 10 μL aliquot

HPLC VARIABLES

Column: μBondapak C18 Radial-Pak in a RCM-100 radial compression module
Mobile phase: Butanol:MeOH:acetic acid:water 8:4:2:86 containing 0.15 mM 1-phenethyl-2-picolinium bromide (Extract 10 mM 1-phenethyl-2-picolinium bromide stock solution with dichloromethane before use to remove UV-absorbing impurities.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: choline, butyrylcholine, propionylcholine

REFERENCE

Jones,R.S.; Stutte,C.A. Chromatographic analysis of choline and acetylcholine by UV visualization, *J.Chromatogr.*, **1985**, *319*, 454–460.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN. B was 0.1% trifluoroacetic acid in water. A:B 90:10 for 1 min, to 70:30 over 17 min, to 0:100 over 2 min, maintain at 0:100 over 4.5 min
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM

Retention time: 3.85

OTHER SUBSTANCES
Simultaneous: choline, sodium, magnesium, calcium

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain tissue with 3 mL 400 mM perchloric acid containing 2 nmoles ethylhomocholine, centrifuge at 35000 g for 20 min, adjust pH of supernatant to about 4.2 with about 200 μL 7.5 M potassium acetate, centrifuge at 35000 g for 20 min. Add the supernatant to 100 μL 5 mM tetramethylammonium chloride, add 3 mL 2% ice-cold reineckate solution, let stand on ice for 1 h, centrifuge at 1000 g at 0° for 10 min. Remove the supernatant and dry the precipitate under vacuum overnight, add about 1 mL 5 mM silver tosylate in MeCN until the pink color disappears, centrifuge at 1000 g at 0° for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL 20 mM pH 3.5 citrate-phosphate buffer, inject an aliquot. Alternatively (for acetylcholinme only), homogenize brain tissue with 400 μL 20 mM pH 3.5 citrate-phosphate buffer, add 4.8 nmoles ethylhomocholine, boil for 10 min, add 40 μL 250 mM zinc sulfate while vortexing, add 40 μL 250 mM barium hydroxide while vortexing, centrifuge at 35000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: ODS-5 (Bio-Rad)
Column: 150 mm long Bio-Sil ODS-5S (Bio-Rad)
Mobile phase: Buffer (Buffer was 10 mM sodium acetate buffered to pH 5 with 20 mM citric acid, containing 4.5 mg/L sodium octyl sulfate and 1.2 mM tetramethylammonium chloride.)
Flow rate: 0.8
Injection volume: 20
Detector: E, Bio Analytical Systems LC-4A, Pt electrode +0.5 V, Ag/AgCl reference electrode following post-column reaction detection. The column effluent mixed with 1 U/mL choline oxidase and 2 U/mL acetylcholinesterase in 200 mM pH 8.5 phosphate buffer pumped at 0.5 mL/min, the mixture flowed through a 30 m × 0.3 mm i.d. PTFE tube (2.5 min) to the detector

CHROMATOGRAM
Retention time: 10
Internal standard: ethylhomocholine (7.2)
Limit of detection: 1 pmole

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
post-column reaction; rat; brain

REFERENCE
Potter,P.E.; Meek,J.L.; Neff,N.H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection, *J.Neurochem.*, **1983**, *41*, 188–194.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate 250 mg rat brain tissue with 6 mL 1 M formic acid containing 10 nmoles IS for 5 min, centrifuge at 4° at 10000 g for 20 min, add the supernatant

to an equal volume of diethyl ether, add 5 mL water, shake, centrifuge at 2000 g for 5 min, discard the organic layer. Lyophilize the aqueous layer, dissolve the residue in 400 μL water, filter (0.45 μm). Add 30 μL reagent to the filtrate, mix, centrifuge at 10000 g for 5 min. Dissolve the precipitate in 300 μL water, add 50 mg Dowex 1x8, shake, centrifuge at 10000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Reagent contained 20% KI and 18% iodine in water.)

HPLC VARIABLES

Column: 150 × 4.6 Nucleosil C18

Mobile phase: Buffer (Prepare buffer by dissolving 1.36 g sodium acetate. 3.72 disodium EDTA, 25 mg sodium octyl sulfate, and 1.2 mmoles tetramethylammonium chloride in 900 mL water, adjust pH to 5.0 with 200 mM citric acid, make up to 1 L.)

Column temperature: 37

Flow rate: 0.8

Injection volume: 10

Detector: E, Bioanalytical Systems LC-4B/17, TL-10A platinum electrode +500 mV, Ag/AgCl reference electrode following post-column reaction. The effluent from the column mixed with buffer pumped at 0.5 mL/min and the mixture flowed through an immobilized enzyme reactor to the detector. (Prepare buffer by dissolving 71.6 g $Na_2HPO_4.12H_2O$ and 372 mg disodium EDTA in 900 mL water, adjust pH to 8.5 with NaH_2PO_4, make up to 1 L. Prepare reactor by heating 200-400 mesh porous glass beads (pore size 400 Å, Electronucleonics, Fairfield NJ) in 5% nitric acid, wash with water, dry, add to 10% 3-aminopropyltriethoxysilane in toluene, reflux overnight. Suspend the beads in 2% glutaraldehyde in water at room temperature for 2 h. Dissolve 0.45 mg acetylcholinesterase (type III, EC.3.1.1.7, Sigma) and 16.6 mg choline oxidase (EC.1.1.3.17, Sigma) in 200 μL 50 mM pH 7.0 phosphate buffer containing 10 mM disodium EDTA, add 500 mg activated beads, pack in a 10 × 4 tube.)

CHROMATOGRAM

Retention time: 10

Internal standard: ethylhomocholine (Prepare ethylhomocholine by adding 3-dimethylamino-1-propanol to EtOH, add bromoethane. When reaction is complete add ether, filter off the precipitate and wash it with ether.) (8)

Limit of detection: 100 fmoles

OTHER SUBSTANCES

Extracted: choline

KEY WORDS

post-column reaction; rat; brain; immobilized enzyme reactor

REFERENCE

Asano,M.; Miyauchi,T.; Kato,T.; Fujimori,K.; Yamamoto,K. Determination of acetylcholine and choline in rat brain tissue by liquid chromatography/electrochemistry using an immobilized enzyme post column reactor, *J.Liq.Chromatogr.*, **1986**, *9*, 199–215.

SAMPLE

Matrix: tissue

Sample preparation: Sonicate rat brain with ten volumes of 1 M formic acid:acetone 15:85 containing IS, centrifuge at 4° at 20000 g. Remove a 500 μL aliquot of the supernatant and add it to 2 mL heptane:chloroform 80:10, vortex. Remove the aqueous layer and add it to 250 μL 3 mg/mL sodium tetraphenylboron in 3-heptanone, vortex. Remove a 200 μL aliquot of the upper organic layer and add it to 50 μL 1 M HCl, vortex. Remove the aqueous layer and evaporate it to dryness under reduced pressure, reconstitute with mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Waters)

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: 100 mM pH 7 KH_2PO_4 containing 10 μg/mL sodium octane sulfate and 600 μg/mL tetramethylammonium chloride
Flow rate: 1
Injection volume: 30
Detector: E, Chromatofield, Pt electrode +0.5 V following post-column reaction. The column effluent flowed through an immobilized enzyme reactor to the detector. (Prepare reactor by heating 200-400 mesh porous glass beads (pore size 350 Å, Sigma) in 5% nitric acid at 100° for 1 min, wash with water, dry, add to 10% 3-aminopropyltriethoxysilane in toluene, heat at 115° for 12 h. Suspend the beads in 2% glutaraldehyde in water at room temperature for 2 h. Dissolve 100 U acetylcholinesterase (type III, electric eel, Sigma) and 100 U choline oxidase (Alcaligenes, Sigma) in 1 mL 50 mM pH 7 phosphate buffer, add 120 mg activated beads, shake periodically, pack in a 20 × 2 tube.)

CHROMATOGRAM

Retention time: 9.5
Internal standard: ethylhomocholine bromide (Prepare ethylhomocholine by adding 3-dimethylamino-1-propanol to EtOH, add bromoethane, when reaction is complete add ether, filter off the precipitate and wash it with ether.) (7)

OTHER SUBSTANCES

Extracted: choline

KEY WORDS

rat; brain; post-column reaction; immobilized enzyme reactor

REFERENCE

Beley,A.; Zekhnini,A.; Lartillot,S.; Fage,D.; Bralet,J. Improved method for determination of acetylcholine, choline, and other biogenic amines in a single brain tissue sample using high performance liquid chromatography and electrochemical detection, *J.Liq.Chromatogr.*, **1987**, *10*, 2977–2992.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize brain tissue with 1 mL 50 mM perchloric acid containing 10 nmoles ethylhomocholine for 1 min (Nissei Model US-300T, 300 W, 20 kHz), centrifuge at 20000 g at 4° for 15 min, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 10 × 4 glassy carbon particles IRICA Type CP-2250 (IRICA Instruments) (removes interfering catecholamines but is not essential)
Column: 60 × 4 3 μm Acetylcholine Separation polymeric styrene-based column (Bioanalytical Systems)
Mobile phase: 50 mM pH 8.4 phosphate containing 1 mM disodium EDTA and 0.4 mM sodium 1-octanesulfonate
Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: E, Bioanalytical systems LC-4A, dual platinum electrodes + 0.50 V, Ag/AgCl reference electrode, following a 5 × 4 reactor containing immobilized acetylcholinesterase and choline oxidase

CHROMATOGRAM

Retention time: 7.8
Internal standard: ethylhomocholine (4.07)

OTHER SUBSTANCES

Extracted: choline

KEY WORDS
rat; brain

REFERENCE
Ikarashi,Y.; Iwatsuki,H.; Blank,C.L.; Maruyama,Y. Glassy carbon pre-column for direct determination of acetylcholine and choline in biological samples using liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *575*, 29–37.

SAMPLE
Matrix: tissue
Sample preparation: Mix brain or heart tissue with 2 mL 6% trichloroacetic acid, homogenize for 10 min at 0°, let stand in an ice bath for 10 min, add 2 mL 100 mM pH 7.4 sodium phosphate, mix, let stand in an ice bath for 10 min, centrifuge at 30000 g for 25 min, dilute the supernatant ten-fold with 100 mM pH 7.4 sodium phosphate, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-8
Column: 250 × 4 5 μm Supelcosil LC-8
Mobile phase: 100 mM pH 7.4 phosphate buffer containing 4 mM tetramethylammonium perchlorate and 0.1 mM EDTA
Flow rate: 0.5
Injection volume: 50
Detector: E, Biometra Model EP 30, 0.45 V following a 30 × 2.1 cartridge with immobilized acetylcholinesterase and cholinoxidase (Biometra)

CHROMATOGRAM
Retention time: 14
Limit of detection: <5 pmole

KEY WORDS
brain; heart

REFERENCE
Salamoun,J.; Nguyen,P.T.; Remien,J. Cation-exchange liquid chromatography of choline and acetylcholine on free shielded silanols of silica-based reversed-phase stationary phases, *J.Chromatogr.*, **1992**, *596*, 43–49.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain tissue with 10 volumes 400 mM perchloric acid, centrifuge. Remove the supernatant and add it to one tenth the volume of 7.5 M potassium acetate solution, centrifuge. Remove a 100 μL aliquot and take it to dryness in a vacuum centrifuge, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 60 × 4.6 5 μm Bakerbond Sulfopropyl
Mobile phase: 100 mM pH 7.5 sodium phosphate with 5 mM tetramethylammonium chloride
Flow rate: 1.2
Detector: E, Biometra EP 20, platinum electrode + 0.5 V following an immobilized enzyme reactor containing choline oxidase (EC 1.1.3.17) and acetylcholine esterase (EC 3.1.1.7) to convert acetylcholine and choline to hydrogen peroxide which was then detected

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 0.3 pmol

OTHER SUBSTANCES
Extracted: choline

KEY WORDS
rat; brain

REFERENCE
Klein,J.; Gonzalez,R.; Köppen,A.; Löffelholz,K. Free choline and choline metabolites in rat brain and body fluids: sensitive determination and implications for choline supply to the brain, *Neurochem.Int.*, **1993**, *22*, 293–300.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Nissei US-300T ultrasonic cell disrupter at 300 W and 20 kHz for 1 min) rat brain striatal tissue with 1 mL 1 μM ethylhomocholine in 50 mM perchloric acid, centrifuge at 4° at 20000 g for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 74-149 μm plastic formed carbon (details in paper)
Column: 60 × 4 3 μm Acetylcholine Separation polymeric styrene column (Bioanalytical Systems)
Mobile phase: 50 mM pH 8.40 Phosphate buffer containing 1 mM disodium EDTA and 0.40 mM sodium 1-octanesulfonate
Column temperature: 35 ± 1
Flow rate: 0.7
Injection volume: 10
Detector: E, Bioanalytical Systems LC-4A, dual Pt working electrode +500 mV, Ag/AgCl reference electrode following post-column reaction. The effluent from the column flowed through a 5 × 4 immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (Bioanalytical Systems) to the detector.

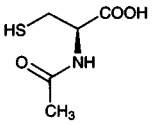
CHROMATOGRAM
Retention time: 12.5
Internal standard: ethylhomocholine (6.5)

OTHER SUBSTANCES
Extracted: choline
Noninterfering: 3,4-dihydroxybenzylamine, dopamine, norepinephrine

KEY WORDS
rat; brain; guard-column removes interferences from catecholamines; post-column reaction; immobilized enzyme reactor

REFERENCE
Ikarashi,Y.; Blank,C.L.; Suda,Y.; Kawakubo,T.; Maruyama,Y. Application of a novel, plastic formed carbon as a precolumn packing material for the liquid chromatographic determination of acetylcholine and choline in biological samples, *J.Chromatogr.A*, **1995**, *718*, 267–272.

Acetylcysteine



Molecular formula: $C_5H_9NO_3S$
Molecular weight: 163.20
CAS Registry No.: 616-91-1
Merck Index: 89

SAMPLE

Matrix: blood

Sample preparation: Add 90 μL cold 10% trichloroacetic acid (containing 1 mM disodium EDTA) to 10 μL blood. Centrifuge at 1850 g at 0° for 5 min. Dilute a 10 μL aliquot of the supernatant with 2 mL water. Mix a 200 μL aliquot of the supernatant with 100 μL 100 μM o-phthalaldehyde reagent and 100 μL 200 μM N-(4-aminobutyl)-N-ethylisoluminol reagent, vortex thoroughly. Let stand for about 2 min. Inject a 20 μL aliquot of the reaction mixture. (Prepare reagents as follows. Dissolve o-phthalaldehyde in 50 mM pH 9.0 sodium borate buffer containing 100 mM potassium dihydrogen phosphate to give a 100 μM solution. Prepare a 1 mM solution of N-(4-aminobutyl)-N-ethylisoluminol (chemiluminescence-grade, Tokyo Kasei) in MeOH containing 5 mM HCl. Dilute with MeOH to give a 200 μM solution.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrosorb RP-18
Column: 150 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque)
Mobile phase: MeOH:THF:100 mM pH 7.5 phosphate buffer 25:5:70
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence, TOA Electronics ICA-3070 detector following post-column reaction. The column effluent mixed with 150 mM hydrogen peroxide pumped at 0.2 mL/min and 25 μM hematin in 150 mM sodium carbonate buffer pumped at 3 mL/min and this mixture flowed through a 200 × 0.5 mm PTFE reaction coil to the detector.

CHROMATOGRAM

Retention time: k' 3.1
Limit of detection: 3.5 nM

OTHER SUBSTANCES

Extracted: captopril

KEY WORDS

post-column reaction; derivatization

REFERENCE

Sano,A.; Nakamura,H. Chemiluminescence detection of thiols by high-performance liquid chromatography using o-phthalaldehyde and N-(4-aminobutyl)-N-ethylisoluminol as precolumn derivatization reagents, *Anal.Sci.*, **1998**, *14*, 731–735.

SAMPLE

Matrix: blood

Sample preparation: Vortex 750 μL red blood cells with 3.2 mL 100 mM HCl, centrifuge at 4° at 15000 rpm for 5 min. Remove the supernatant and mix it with an equal volume of cold 4 M sodium methanesulfonate, freeze in dry ice/isopropanol, thaw, centrifuge at 20000 rpm for 10 min. Determine the thiol content of the supernatant by titrating with 5,5'-dithiobis(2-nitrobenzoic acid). Add N-ethylmorpholine to a final concentration of 10 mM, adjust pH to 8.0 with 1 M NaOH, add a 1 molar equivalent of dithiothreitol, mix, let stand at room temperature for 5 min, add 6 equivalents of monobromotrimethylam-

moniobimane, mix, let stand for 15 min, add 12 equivalents of thiol agarose, let stand for 20 min, add acetic acid to a final concentration of 3%, inject an aliquot. (Prepare 4 M sodium methanesulfonate by adjusting the pH of methanesulfonic acid to 1.5 with 50% NaOH then diluting to 4 M.)

HPLC VARIABLES

Column: 150 × 4 AA-10 resin (Beckman)

Mobile phase: Gradient. A was 2-methoxyethanol:200 mM pH 3.20 buffer 10:90 at 45°. B was 2-methoxyethanol:200 mM pH 4.40 buffer 10:90 at 45°. C was 2-methoxyethanol:200 mM pH 4.75 buffer 10:90 at 45°. D was 2-methoxyethanol:0.2 N pH 6.40 trisodium citrate containing 800 mM NaCl 10:90 at 55°. E was 2-methoxyethanol:100 mM NaOH containing 100 mM NaCl 10:90 at 55°. A for 10 min; B for 20 min; C for 5 min, D for 90 min, E for 10 min, re-equilibrate at initial conditions for 20 min.

Flow rate: 0.2

Detector: F (o-phthalaldehyde filters)

CHROMATOGRAM

Retention time: 27.5

Limit of detection: 1 pmole

Limit of quantitation: 10 pmole

OTHER SUBSTANCES

Extracted: coenzyme A, coenzyme M, cysteine, cysteinylglycine, dithiothreitol, ergothioneine, gamma-glutamylcysteine, glutathione, homocysteine, 2-mercaptoethanol, methanethiol, pantetheine, 4'-phosphopantetheine, thiosulfate, thiouracil

KEY WORDS

derivatization; red blood cells

REFERENCE

Fahey,R.C.; Newton,G.L.; Dorian,R.; Kosower,E.M. Analysis of biological thiols: Quantitative determination of thiols at the picomole level based upon derivatization with monobromobimanes and separation by cation-exchange chromatography, *Anal.Biochem.*, **1981**, *111*, 357–365.

SAMPLE

Matrix: blood

Sample preparation: Vortex 750 μL red blood cells with 3.2 mL 100 mM HCl, centrifuge at 4° at 15000 rpm for 5 min. Remove the supernatant and mix it with an equal volume of cold 4 M sodium methanesulfonate, freeze in dry ice/isopropanol, thaw, centrifuge at 20000 rpm for 10 min. Determine the thiol content of the supernatant by titrating with 5,5'-dithiobis(2-nitrobenzoic acid). Add N-ethylmorpholine to a final concentration of 10 mM, adjust pH to 8.0 with 1 M NaOH, add a 1 molar equivalent of dithiothreitol, mix, let stand at room temperature for 5 min, add 6 equivalents of monobromobimane, mix, let stand for 15 min, add 12 equivalents of thiol agarose, let stand for 20 min, add acetic acid to a final concentration of 3% (Anal. Biochem. 1981, 111, 357), dilute with 200 mM pH 2.2 sodium citrate, inject an aliquot. (Prepare 4 M sodium methanesulfonate by adjusting the pH of methanesulfonic acid to 1.5 with 50% NaOH then diluting to 4 M.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere-ODS C18

Mobile phase: Gradient. A was MeOH:water:acetic acid 10:89.75:0.25, adjusted to pH 3.9 with 50% NaOH. B was MeOH:water:acetic acid 90:9.75:0.25, adjusted to pH 3.9 with 50% NaOH. A:B 92:8 for 10 min, to 60:40 over 10 min, maintain at 60:40 for 5 min, to 10:90 over 5 min, to 0:100 over 2 min.

Flow rate: 1.5

Detector: F (o-phthalaldehyde filters)

CHROMATOGRAM
Retention time: 11.8
Limit of detection: 2-20 pmole

OTHER SUBSTANCES
Extracted: coenzyme A, coenzyme M, cysteamine, cysteine, cysteinylglycine, ergothioneine, ethanethiol, gamma-glutamylcysteine, glutathione, homocysteine, hydrogen sulfide, 2-mercaptoethanol, mercaptopyrimidine, methanethiol, pantetheine, 4'-phosphopantetheine, thiosulfate, 2-thiouracil

KEY WORDS
derivatization; red blood cells

REFERENCE
Newton,G.L.; Dorian,R.; Fahey,R.C. Analysis of biological thiols: derivatization with monobromobimane and separation by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1981**, *114*, 383–387.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 200 g/L trichloroacetic acid, vortex, let stand for 15 min, centrifuge. Remove 600 μL supernatant and add it to 400 μL 20 mM disodium EDTA and 1 mL 240 mM pH 10.0 borate buffer, add 1 mL of Thiopropyl-Sepharose 6B (containing 20 μmol thiol per mL of gel suspension) (Pharmacia), place in an end-over-end mixer for 30 min, add 1 mL 4 M acetic acid, centrifuge. Add 3 mL of the supernatant to a 13 × 7 column of p-acetoxymercurianiline-Sepharose 4B (Biochim.Biophys.Acta 1970, 200,593), wash with two 1 mL portions of water, elute with 3 mL 10 mM cysteine hydrochloride. Add the eluate followed by 1 mL 10 mM HCl to a 25 × 5 AG 50W-X8 (H+) 100-200 mesh column (Bio-Rad). Collect the effluents and add them to 200 μL 180 mM disodium EDTA. Remove a 2 mL aliquot and add it to 200 μL 100 mM NaOH, add 3 mL 50 mM pH 9.0 carbonate buffer containing 10 mM disodium EDTA, add 500 μL 20 μM N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in acetone, heat at 37° overnight, dilute 1:5 with mobile phase, inject a 100 μL aliquot onto column A. When the acetylcysteine has eluted from column A to column B remove column A from the circuit, monitor the effluent from column B. Backflush column A with mobile phase to clean it.

HPLC VARIABLES
Column: A 30 × 4.6 10 μm 10 μm Brownlee RP-8; B 250 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeOH:2 mM sodium phosphate 15:85, containing 10 mM tetramethylammonium hydroxide, pH adjusted to 7.4 with 6 M HCl
Flow rate: 0.75
Injection volume: 100
Detector: F ex 360 (filter) em 418-700 (filter)

CHROMATOGRAM
Retention time: 15

KEY WORDS
plasma; column-switching; SPE

REFERENCE
Kagedal,B.; K:allberg,M.; Martensson,J. Determination of non-protein-bound N-acetylcysteine in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *311*, 170–175.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Plasma + 5 μL 100 mM dithiothreitol in 10% Triton X-100, vortex, allow to stand for 30 min, add 100 μL 30 mM monobromobimane (Calbiochem; Molecular Probes) in 50 mM pH 8.0 N-ethylmorpholine, store in the dark for 5 min, add 10 μL 100% (w/v) trichloroacetic acid, centrifuge at 3000 g for 3 min, inject a 25 μL aliquot of the supernatant. (Dissolve monobromobimane in the minimum amount of MeCN before making up aqueous solutions.)(J. Biochem. Biophys. Methods 1986, 13, 231)

HPLC VARIABLES

Column: 75 × 4.5 3 μm Supelco octadecylsilica
Mobile phase: Gradient. A was MeCN:acetic acid:perchloric acid:water 9:0.25:0.25:90.75, pH 3.7. B was MeCN:water:perchloric acid 75:25:0.25. A:B 100:0 for 7 min then 0:100 for 4 min then re-equilbrate at 100:0 for 7 min.
Flow rate: 1
Injection volume: 25
Detector: F ex 394 em 480

CHROMATOGRAM

Retention time: 7.4
Limit of detection: 0.5 nM

KEY WORDS

plasma; derivatization

REFERENCE

Cotgreave,I.A.; Moldéus,P. Methodologies for the analysis of reduced and oxidized N-acetylcysteine in biological systems, *Biopharm.Drug Dispos.*, **1987**, *8*, 365–375.

SAMPLE

Matrix: blood, tissue
Sample preparation: Tissue. Homogenize (Potter-Elvehjem PTFE-glass homogenizer) tissue in 20 mM EDTA, adjust to 1% (w/v) (kidney) or 2.5% (w/v) (spleen). Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Plasma. Dilute rat plasma to 20% (v/v) with 20 mM EDTA. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Serum. Dilute human serum to 10% (v/v) with 20 mM EDTA. 1 mL Diluted serum + 200 μL 30% metaphosphoric acid, centrifuge at 2000 g at 4° for 20 min. Remove 500 μL of the supernatant and add it to 240 μL 2 M KOH. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. (Synthesis of N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide is as follows. Add 8.8 g aluminum trichloride to 12.50 g 3-dimethylaminophenol in 185 mL chloroform and 84 g triethyl orthoformate, mix at room temperature for 10 min, when the exothermic reaction ceases add 50 mL 10% HCl, stir to hydrolyze the acetal, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform, wash the filtrate with saturated aqueous NaCl, dry over magnesium sulfate, concentrate under reduced pressure, recrystallize from chloroform to give 4-(dimethylamino)salicylaldehyde (mp 78-79°). Add 400 mg KOH in 3 mL EtOH to a solution of 1 g 4-(dimethylamino)salicylaldehyde and 1.3 g (?) 4-nitrobenzylbromide in 12 mL EtOH, reflux for 7 h, cool, filter to recover the crystals, wash with water, dry under vacuum, recrystallize from EtOH to give 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde (mp 179-180°). Add a solution of 900 mg 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde in 6 mL DMF to a sodium methoxide

solution (prepared from 69 mg sodium in 1 mL MeOH), reflux for 20 min, add 1 mL MeOH, filter the crystals, recrystallize from EtOH to give 6-dimethylamino-2-(4-nitrophenyl)benzofuran as red needles (mp 209.5-210.5°). Reflux 1 g 6-dimethylamino-2-(4-nitrophenyl)benzofuran in 20 mL benzene (Caution! Benzene is a carcinogen!) and 18 mL MeOH containing 80 mg active carbon and a catalytic amount of ferric chloride hexahydrate for 10 min, add 2.30 g 98% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) dropwise, reflux for 7 h, filter, concentrate the filtrate, recrystallize from cyclohexane to give 6-dimethylamino-2-(4-aminophenyl)benzofuran as orange needles (mp 198.5-200°). Stir 605 mg 6-dimethylamino-2-(4-aminophenyl)benzofuran and 230 mg maleic anhydride in 5 mL chloroform at room temperature for 3 h, filter the crystals, wash with a small amount of chloroform, recrystallize from EtOH to obtain N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid (mp 219.5-221°). Reflux a mixture of 1.17 g N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid and 30 mg sodium acetate in 18 mL acetic anhydride, cool in an ice bath, collect the crystals of product, wash with water. Neutralize the filtrate with 20% NaOH, extract twice with 30 mL portions of chloroform, wash the organic layers with saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to give more product. Combine the products and recrystallize them from acetone to give N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide as reddish purple crystals (mp 203-204°) (Bull.Chem.Soc.Jpn. 1985, 58, 2192).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Toyo Soda ODS-80
Mobile phase: MeCN:10 mM pH 7.7 phosphate buffer 47.5:52.5 containing 30 mM tetrabutylammonium bromide
Flow rate: 0.8
Injection volume: 20
Detector: F ex 355 em 457

CHROMATOGRAM

Retention time: 6
Internal standard: disodium 6-amino-1,3-naphthalene disulfonate (3.5)
Limit of detection: 20 fmole

OTHER SUBSTANCES

Extracted: homocysteine, reduced glutathione (GSH), cysteamine, cysteine, coenzyme A

KEY WORDS

plasma; serum; rat; human; liver; kidney; spleen; derivatization

REFERENCE

Nakashima,K.; Umekawa,C.; Yoshida,H.; Nakatsuji,S.; Akiyama,S. High-performance liquid chromatography-fluorometry for the determination of thiols in biological samples using N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]-maleimide, *J.Chromatogr.*, **1987**, *414*, 11–17.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition CF50A Centriflo ultrafiltration cones (Amicon) by immersing in water for 1 h and centrifuging at 1000 g for 5 min. 3 mL Plasma + 1 mL water + 500 μL 5 mg/mL dithiothreitol, vortex for 10 s, incubate at 37° for 30 min, add 1 mL 2% sodium bicarbonate, add 350 μL 5% 2,4-dinitro-1-fluorobenzene in EtOH, vortex for 10 s, incubate at 60° for 30 min, place in ultrafiltration cone, centrifuge at 1000 g at 20° for 20 min. Remove 1 mL of the ultrafiltrate and add it to 5 mL water and 10 mL ether, shake mechanically in the dark at 250 cycles/min for 5 min, centrifuge at 1000 g at 10° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 75 μL mobile phase. Urine. 100 μL urine + 1 mL water + 500 μL 5 mg/mL dithiothreitol, vortex for 10 s, incubate at 37° for 30 min, add 1 mL 2% sodium bicarbonate, add 350 μL 5% 2,4-dinitro-1-fluorobenzene in EtOH, vortex for 10 s, incubate at 60° for 30 min. Remove 1 mL and add it to 5 mL water and 10 mL

ether, shake mechanically in the dark at 250 cycles/min for 5 min, centrifuge at 1000 g at 10° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:50 mM trisodium citrate and 1 mM EDTA adjusted to pH 7.0 with citric acid solution 30:70 (plasma) or 35:65 (urine)
Flow rate: 1
Injection volume: 50
Detector: UV 360

CHROMATOGRAM
Retention time: 13
Limit of detection: 50 ng/mL

KEY WORDS
plasma; derivatization; ultrafiltration

REFERENCE
Lewis,P.A.; Woodward,A.J.; Maddock,J. High-performance liquid chromatographic assay for N-acetylcysteine in plasma and urine, *J.Pharm.Sci.*, **1984**, *73*, 996–998.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out tablets, capsule contents, or granules equivalent to 50 mg N-acetylcysteine, add 10 mL IS solution, add 80 mL 0.05% sodium hydrogen sulfite, make up to 100 mL with MeOH, inject a 10 μL aliquot. (IS solution was 250 mg methionine in 40 mL 0.05% sodium hydrogen sulfite, adjusted to pH 3 with phosphoric acid, sonicate, make up to 50 mL with MeOH.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak ODS
Mobile phase: MeCN:MeOH:buffer 0.8:0.6:98.6 (Buffer was 5 mM sodium hexanesulfonate adjusted to pH 2.9 with phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 6.8
Internal standard: methionine (11)

OTHER SUBSTANCES
Simultaneous: S-carboxymethylcysteine, methylcysteine

KEY WORDS
tablets; capsules; granules

REFERENCE
Tsai,F.Y.; Chen,C.J.; Chien,C.S. Determination of the cysteine derivatives N-acetylcysteine, S-carboxymethylcysteine and methylcysteine in pharmaceuticals by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *697*, 309–315.

SAMPLE
Matrix: formulations

Sample preparation: Dissolve solutions, powders, or powdered tablets in water to give a 200-325 μM solution, filter if necessary. Mix 500 μL solution with 500 μL 170 mM pH 7.5 borate buffer and 500 μL 2 mM 1,1-bis(phenylsulfonyl)ethylene (1,1'-ethenylidene-bis(sulfonyl)bis-benzene; Fluka, Merck) in MeOH, let stand at room temperature for 2 min, add 500 μL water, add 300 μL chloroform, vortex for 1 min, centrifuge for 2 min. Remove a 1 mL aliquot of the aqueous layer and add it to 500 μL 300 mM orthophosphoric acid, add 100-200 μL 30 μM IS in MeCN, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 36:64 (Buffer was 50 mM pH 4.0 triethylamine-phosphate buffer.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Internal standard: methyl p-hydroxybenzoate (10)
Limit of detection: 100 pmole

KEY WORDS

derivatization; tablets; powders

REFERENCE

Cavrini,V.; Gotti,R.; Andrisano,V.; Gatti,R. 1,1'-[Ethylidenebis(sulfonyl)]bis-benzene: A useful pre-chromatographic derivatization reagent for HPLC analyses of thiol drugs, *Chromatographia*, **1996**, *42*, 515–520.

SAMPLE

Matrix: solutions
Sample preparation: Mix 330 μL 3 mM 4-chloro-7-sulphobenzofurazan in 100 mM pH 9.2 borate buffer with 100 μL 1 M NaOH and 230 μL water containing excess of thiol (13.3 fold), after the appearance of a yellow color add 100 μL 1 M HCl, dilute to 1330 μL with 200 mM pH 7 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 3 μm Nova-Pak C18
Mobile phase: MeCN:150 mM H_3PO_4 5:95 adjusted to pH 2.5
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 510

CHROMATOGRAM

Retention time: 8.01

OTHER SUBSTANCES

Simultaneous: cysteinylglycine, reduced glutathione, cysteine

REFERENCE

Chen,X.; Cross,R.F.; Clark,; Baker,W.L. Chromatographic separation of fluorescent thiol adducts of 4-chloro-7-sulphobenzofurazan. Use as substrates for enzymes of the mercapturic acid xenobiotic pathway, *J.Chromatogr.B*, **1998**, *709*, 19–25.

SAMPLE

Matrix: solutions

Sample preparation: Add 1 mL 50 μg/mL N-(4-anilinophenyl)maleimide in 33 mM pH 6.85 phosphate buffer to 0.1-4 μg thiol, let stand at 0° for 90 min, wash twice with 2 mL portions of ether, heat the aqueous phase to 50° for 20 min, inject an aliquot. (Prepare N-(4-anilinophenyl)maleimide as follows. Add dropwise 1.1 g maleic anhydride in 10 mL chloroform to 1 g N-phenylphenylenediamine (4-aminodiphenylamine) stirred in 10 mL chloroform at 0°, filter, dry to give N-(4-anilinophenyl)maleamic acid. Heat 100 mg N-(4-anilinophenyl)maleamic acid and 25 mg sodium acetate in 400 μL acetic anhydride on a water bath for 2 h, cool, pour into ice-water, filter, recrystallize from ethyl acetate/hexane to give N-(4-anilinophenyl)maleimide as yellow needles (mp 135-6°).)

HPLC VARIABLES

Column: 305 × 6.3 μBondapak C18
Mobile phase: MeCN:0.5% pH 3.0 $(NH_4)H_2PO_4$ 4:7
Flow rate: 1
Injection volume: 10
Detector: E, Yanagimoto model VMD-101, glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: L-cysteine, glutathione, D-penicillamine

KEY WORDS

derivatization

REFERENCE

Shimada,K.; Tanaka,M.; Nambara,T. Sensitive derivatization reagents for thiol compounds in high-performance liquid chromatography with electrochemical detection, *Anal.Chim.Acta*, **1983**, *147*, 375–380.

SAMPLE

Matrix: solutions
Sample preparation: Mix 1 mL of a 2 μM solution of thiols in 100 mM pH 8.0 sodium borate buffer containing 2 mM disodium EDTA with 1 mL 1 mM ABD-F in 100 mM pH 8.0 sodium borate buffer, vortex, heat at 50° for 5 min, cool in ice, add 600 μL 100 mM HCl, inject a 10 μL aliquot. (Synthesis of ABD-F is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is

a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°). Add 1 g CBD-F dropwise to 100 mL 6% ammonium hydroxide, neutralize with 10% HCl, evaporate under reduced pressure, add 200 mL MeCN to the residue, filter. Evaporate the filtrate and chromatograph on a 300 × 20 column of 100-200 mesh silica with chloroform, collect the appropriate fractions and evaporate them to give ABD-F (4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole) as white needles (mp 145-6°) after recrystallization from n-hexane/benzene (Caution! Benzene is a carcinogen!).

HPLC VARIABLES

Guard column: 20 × 3.9 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 8-10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 4.0 potassium biphthalate buffer 8:92
Flow rate: 1
Injection volume: 10
Detector: F ex 380 em 510

CHROMATOGRAM

Retention time: 10
Limit of detection: 1.9 pmole

OTHER SUBSTANCES

Extracted: cysteamine, cysteine, glutathione, homocysteine

KEY WORDS

derivatization

REFERENCE

Toyo'oka,T.; Imai,K. New fluorigenic reagent having halogenobenzofurazan structure for thiols: 4-(Aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole, *Anal.Chem.*, **1984**, *56*, 2461–2464.

SAMPLE

Matrix: solutions

Sample preparation: Mix 400 μL 2.5 μg/mL acetylcysteine in buffer with 100 μL 55 μg/mL N-(ferrocenyl)maleimide in acetone, let stand at 0° for 30 min, wash with three 2 mL portions of diethyl ether:hexane 50:50, inject an aliquot of the aqueous layer. (Buffer was 67 mM pH 6.8 phosphate buffer containing 1 mM EDTA. Prepare N-(ferrocenyl)maleimide as follows. Stir 13 g ferrocene in 200 mL anhydrous THF at -30° under nitrogen, add 160 mL 1.3 M butyllithium in ether dropwise over 25 min, stir at 0° for 2 h, stir at room temperature for 4 h. Stir at -20° and add 10.3 g methoxylamine in 75 mL anhydrous ether dropwise over 30 min, allow to warm gradually to room temperature, stir for 4 h, slowly add 10% HCl with stirring until the pH of the aqueous layer is 2, discard the organic layer. Make the aqueous layer strongly basic with KOH, extract with ether, extract the ether layer with 2 M HCl. Make the aqueous layer basic with KOH, extract with ether, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from ether/petroleum ether to obtain ferrocenylamine (8%, mp 140-145°) (J. Org. Chem. 1959, 24, 1487). Dissolve 300 mg ferrocenylamine in the minimum amount of chloroform, add 150 mg maleic anhydride in a little chloroform. Collect the compound that crystallizes and heat 300 mg with 1.5 g acetic anhydride and 60 mg anhydrous sodium acetate at 50-60° for 3 h with the exclusion of moisture (Chem. Zvesti 1963, 17, 21), recrystallize the product from diethyl ether to give N-(ferrocenyl)maleimide as deep purple prisms (mp 151-152°).

HPLC VARIABLES

Column: 150 × 4.6 5 μm YMC-GEL C8 (Yamamura, Kyoto)
Mobile phase: MeCN:buffer 20:50 (Buffer was 0.32% Na_2HPO_4 adjusted to pH 5.0 with phosphoric acid.)

Flow rate: 1
Detector: E, Environmental Sciences Associates 5100A, 5011 porous graphite dual electrode analytical cell, upstream electrode +150 mV, downstream electrode -100 mV, 5020 guard cell +200 mV, palladium reference electrode

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: L-cysteine, glutathione

KEY WORDS
derivatization

REFERENCE
Shimada,K.; Oe,T.; Nambara,T. Sensitive ferrocene reagents for derivatization of thiol compounds in high-performance liquid chromatography with dual-electrode coulometric detection, *J.Chromatogr.*, **1987**, *419*, 17–25.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL Solution + 300 μL reagent solution, let stand at room temperature for 20 min, add 500 μL 300 mM phosphoric acid solution, make up to 10 mL with water, inject a 50 μL aliquot. (Prepare the reagent solution by dissolving 3.5 mg methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate in 10 mL THF, make up to 25 mL with pH 7.5 borate buffer. Prepare methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as follows. Dissolve 5 g 6'-methoxy-2'-acetonaphthone in warm glacial acetic acid and add 2.5 g glyoxylic acid, reflux for 24 h, evaporate to dryness under reduced pressure. Take up the residue in chloroform and extract it three times with 5% sodium carbonate solution. Combine the aqueous layers and acidify them with concentrated HCl, collect the product by filtration, recrystallize from MeOH/water or acetic acid to give 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid (mp 167-9°) (Farmaco, Ed. Sci. 1982, 37, 171). Reflux 0.5 g 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid, 2.5 mL MeOH, and 2-3 drops sulfuric acid in 25 mL anhydrous benzene (Caution! Benzene is a carcinogen!) for 1 h, add 20 mL water, wash the organic layer with 10 mL 5% sodium bicarbonate solution, wash the organic layer with 20 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, purify by flash chromatography on silica gel using ethyl acetate:light petroleum (bp 40-70°) 40:60 to give methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as a pale yellow compound (mp 116-120°).)

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb RP-8
Mobile phase: MeOH:50 mM pH 3.0 triethylammonium phosphate 53:47
Flow rate: 1
Injection volume: 50
Detector: F ex 310 em 450

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: cysteamine, cysteine, glutathione, homocysteine, mesna
Noninterfering: bacitracin, biotin, calcium pantothenate, cystine, glycine, magnesium oxide, neomycin, starch, threonine, vitamin E, pyridoxine, riboflavin phosphate

KEY WORDS
solutions

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P.; Pinzauti,S. High-performance liquid chromatographic determination of aliphatic thiols with aroylacrylic acids as fluorogenic precolumn derivatization reagents, *J.Chromatogr.*, **1990**, *507*, 451–458.

SAMPLE
Matrix: solutions
Sample preparation: Mix 250 μL of a solution in 50 mM pH 9.3 borate buffer containing 1 mM disodium EDTA with 250 μL 1 mM SAOX-Cl in MeCN, let stand in the dark at room temperature for 1 h, add 500 μL MeCN:1 M HCl 50:50, inject an aliquot. (Prepare SAOX-Cl as follows. Gently reflux 21 g benzoin and 45 g urethane (Caution! Urethane is a carcinogen!) in 300 mL DMF for 6 h, cool, pour into water, filter, recrystallize to give 4,5-diphenyl-2-oxazolone (mp 211°) (Ber. 1956, 89, 1749). Carefully add 60 mL dimethylsulfamoyl chloride (?) to 7.3 g 4,5-diphenyl-2-oxazolone at 0°, heat at 55-60° for 4 h, cool, add dropwise to 500 g ice-water, filter, wash the solid with 4 L water. Add 100 mL dry benzene (Caution! Benzene is a carcinogen!) to 2 g of the crude material (4,5-bis(p-N,N-dimethylaminosulfonylphenyl)-2-oxazolone) and evaporate to dryness to remove traces of moisture, suspend the residue in 30 mL phosphorus oxychloride, stir at 0°, add 610 μL triethylamine dropwise, heat at 100° for 7 h, remove excess phosphorus oxychloride using a rotary evaporator. Dissolve the residue in dichloromethane and wash with cold saturated sodium bicarbonate, dry the organic layer over anhydrous magnesium sulfate, evaporate to dryness, chromatograph on 100 g silica gel with dichloromethane:ethyl acetate 90:10 to give SAOX-Cl (2-chloro-4,5-bis(p-N,N-dimethylaminosulfonylphenyl)oxazole) as a white solid (mp 222-224°) (Analyst 1993, 118, 257).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-8 (Supelco)
Mobile phase: MeCN:100 mM phosphoric acid 35:65
Flow rate: 1
Detector: F ex 330 em 425

CHROMATOGRAM
Retention time: 22.5
Limit of detection: 1.5 fmole

OTHER SUBSTANCES
Simultaneous: captopril, cysteine, glutathione, homocysteine, 2-mercaptopropionylglycine

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Chokshi,H.P.; Givens,R.S.; Carlson,R.G.; Lunte,S.M.; Kuwana,T. Fluorescence and chemiluminescence detection of oxazole-labelled amines and thiols, *Biomed.Chromatogr.*, **1993**, *7*, 208–216.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL N-acetylcysteine solution, 100 μL reagent solution, and 700 μL pH 8.4 borate buffer, vortex for a few s, let stand at room temperature for 1 h, add an equal volume of the mobile phase, inject a 10 μL aliquot. (Prepare reagent, 2-(4-N-maleimidephenyl)-6-methylbenzothiazole, as follows. Recrystallize 2-(4-aminophenyl)-6-methylbenzothiazole from chloroform before use. Add 500 mg maleic anhydride in 2 mL chloroform dropwise to 1.2 g 2-(4-aminophenyl)-6-methylbenzothiazole in 10 mL DMF, stir at room temperature for 2 h, filter, wash with 30 mL chloroform, recrystallize from DMF to give 2-(4-N-phenylmaleamic acid)-6-methylbenzothiazole as yellow crystals (mp 242°). Reflux 2 g 2-(4-N-phenylmaleamic acid)-6-methylbenzothiazole, 100 mg anhydrous sodium acetate, and 25 mL acetic anhydride for 2 h, cool on ice, filter, wash the solid with

water. Neutralize the filtrate with cold 10% NaOH, extract with chloroform. Dry the organic layer over anhydrous magnesium sulfate and evaporate it to dryness under reduced pressure. Combine this product with the solid obtained earlier and recrystallize from isopropanol to give 2-(4-N-maleimidephenyl)-6-methylbenzothiazole as yellow needles (mp 254-6°). Prepare the reagent solution by dissolving 50 μmoles of this compound in 10 mL DMF and diluting 25-fold with MeCN.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 35:65, pH 4.5 (Buffer was 10 mM KH_2PO_4 containing 0.1% sodium hexanesulfonate.)
Flow rate: 1.5
Injection volume: 10
Detector: F ex 320 em 405

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: N-acetylpenicillamine, coenzyme A, cysteine, glutathione, homocysteine, penicillamine

KEY WORDS
derivatization

REFERENCE
Haj-Yehia,A.I.; Benet,L.Z. Determination of aliphatic thiols by fluorometric high-performance liquid chromatography after precolumn derivatization with 2-(4-N-maleimidophenyl)-6-methylbenzothiazole, *Pharm.Res.*, **1995**, *12*, 155–160.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 200 μM solution in buffer with three volumes of a 400 μM solution of 5,5'-dithio-(bis-2-nitrobenzoic acid) in buffer, let stand at room temperature for 30 min, inject a 75 μL aliquot. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS1
Mobile phase: Gradient. MeCN:buffer 0:100 for 20 min, to 17.5:82.5 over 40 min. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)
Flow rate: 0.25 for 20 min, to 1 over 40 min
Injection volume: 75
Detector: UV 357

CHROMATOGRAM
Retention time: 41

OTHER SUBSTANCES
Simultaneous: N-acetylpenicillamine, captopril, cysteine, glutathione, penicillamine, thiomalic acid

KEY WORDS
derivatization

REFERENCE
Russell,J.; McKeown,J.A.; Hensman,C.; Smith,W.E.; Reglinski,J. HPLC determination of biologically active thiols using pre-column derivatization with 5,5'-dithio-(bis-2-nitrobenzoic acid), *J.Pharm. Biomed.Anal.*, **1997**, *15*, 1757–1763.

SAMPLE
Matrix: tissue
Sample preparation: Freeze tissue in liquid nitrogen and pulverize. Homogenize 50-100 mg tissue in 1 mL MeCN:20 mM EDTA 30:70, centrifuge at 4° at 4000 g for 5 min, adjust to 1-2.5% w/v with pH 8.4 borate buffer, keep on ice. 100 μL Sample + 100 μL 0.25 mM reagent in MeCN:DMF 95:5 + 700 μL pH 8.4 borate buffer, vortex for a few s, let stand for 1 h at room temperature, dilute with an equal volume of mobile phase, inject a 10 μL aliquot. (Reagent was 2-(4-maleimidophenyl)-6-methoxybenzofuran, a partial synthesis is given in the paper.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere-ODS
Mobile phase: MeCN:buffer 35:65 adjusted to pH 4.5 (Buffer was 10 mM KH_2PO_4 containing 0.1% sodium hexanesulfonate.)
Flow rate: 1.5
Injection volume: 10
Detector: F ex 310 em 390

CHROMATOGRAM
Retention time: 8
Internal standard: N-acetylcysteine

OTHER SUBSTANCES
Extracted: glutathione, homomocysteine, penicillamine, acetylpenicillamine

KEY WORDS
rat; heart; lung; liver; kidney; testes; spleen; N-acetylcysteine is IS

REFERENCE
Haj-Yehia,A.I.; Benet,L.Z. 2-(4-N-Maleimidophenyl)-6-methoxybenzofuran: a superior derivatizing agent for fluorimetric determination of aliphatic thiols by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *666*, 45–53.

SAMPLE
Matrix: urine
Sample preparation: Add 10 μL 50 μm 2-mercaptoethanol and 100 μL 10% trichloroacetic acid containing 10 mM EDTA to 100 μL urine, centrifuge at 760 g at 4° for 10 min. Add 350 μL 1 M pH 10.5 potassium borate buffer, 100 μL 1% tri-n-butylphosphine in water, and 100 μL 0.3% ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate in water to a 150 μL aliquot of the supernatant yielding a final pH of about 8.5. Incubate the mixture at 60° for 60 min, then put in an ice bath and add 50 μL 4 M HCl, inject a 10 μL aliquot of this solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil 5C-18AR (Nakarai Tesque, Japan)
Mobile phase: MeOH:75 mM pH 2.9 sodium citrate buffer 2:98
Flow rate: 1
Injection volume: 10
Detector: F ex 386 em 516

CHROMATOGRAM
Retention time: 27.5

Internal standard: 2-mercaptoethanol

OTHER SUBSTANCES

Simultaneous: cysteamine, cysteine, homocysteine, cysteinylglycine, γ-glutamylcysteine, glutathione, homocysteine

KEY WORDS

derivatization; mouse

REFERENCE

Oe,T.; Ohyagi,T.; Naganuma,A. Determination of γ-glutamylglutathione and other low-molecular-mass biological thiol compounds by isocratic high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1998**, *708*, 285–289.

SAMPLE

Matrix: urine

Sample preparation: Mix 5 mL urine with 200 μL 130 mM disodium EDTA, adjust pH to 9.8-10.0 with 5 M ammonia, make up to 6 mL with water, add 1 mL thiopropyl-Sepharose 6-B suspension, shake mechanically for 30 min, acidify to pH 3.5-4.0 with 1 mL 4 M acetic acid, centrifuge, add a 3-5 mL aliquot of the supernatant (containing up to 2 μmoles thiol) to a 13 × 7 p-acetoxymercurianiline Sepharose 4-B column, wash with 2 mL water, elute with 3 mL 10 mM cysteine hydrochloride. Add the eluate to a 25 × 5 column of 100-200 mesh AG 50 W-X8 (hydrogen form, Bio-Rad), elute with 1 mL 10 mM HCl, collect all the effluent (Clin. Chim. Acta 1979, 95, 189), add 200 μL 180 mM disodium EDTA. Remove a 250 μL aliquot and neutralize it with 25 μL 100 mM NaOH, add 5 mL 50 mM pH 9.0 carbonate buffer containing 10 mM disodium EDTA, add 500 μL 500 μM N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in acetone, heat at 37° for 20 h, dilute 1:5 with mobile phase, inject a 100 μL aliquot. (Thiopropyl-Sepharose 6-B suspension contains 20 μmoles thiol/mL. Before use convert to free thiol form with dithiothreitol according to the manufacturer's instructions. Prepare p-acetoxymercurianiline-Sepharose 4B as follows. Mix 100 g Sepharose 4B with an equal volume of water, for each 1 mL Sepharose add 100 mg cyanogen bromide in an equal volume of water, adjust pH to 11 with 4 M NaOH, maintain at pH 11 with 4 M NaOH (Proc. Natl. Acad. Sci. USA 1968, 61, 636), at the end of the reaction (about 8 min), wash with 1.5 L 100 mM pH 9.0 sodium bicarbonate. Suspend in 100 mL DMSO:water 10:90 at 0°, slowly add 1.3 g 4-aminophenylmercuric acetate in 20 mL DMSO, stir slowly at 0° for 20 h, warm to 30°, filter, resuspend in 130 mL DMSO:water 20:80 at 35° for 5 min, filter, repeat this procedure 4 times, pack in a column, slowly wash with DMSO:water 20:80 until no mercury appears in the effluent (about 500 mL), store as a slurry in DMSO:water 20:80 (Biochim. Biophys. Acta 1970, 200, 593).)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8

Mobile phase: MeOH:buffer 15:85 (Buffer was 2 mM sodium phosphate buffer containing 11.8 mM tetramethylammonium hydroxide, pH adjusted to 7.4 with 6 M HCl.)

Flow rate: 0.75

Injection volume: 100

Detector: F ex 400 em 480

CHROMATOGRAM

Retention time: 20

Limit of detection: 53 fmole

OTHER SUBSTANCES

Extracted: mercaptoacetate

KEY WORDS

derivatization; SPE

REFERENCE

Kågedal,B.; Källberg,M. Reversed-phase ion-pair high-performance liquid chromatography of mercaptoacetate and N-acetylcysteine after derivatization with N-(1-pyrene)maleimide and N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide, *J.Chromatogr.*, **1982**, *229*, 409–415.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + EDTA, adjust pH to 9.8-10.0, react with thiopropyl-Sepharose, acidify with acetic acid, centrifuge. Remove a portion of the supernatant containing no more than 2 μmoles of thiols and add it to p-acetoxymercurianiline-Sepharose 4-B (Biochim. Biophys. Acta 1970, 200, 593), wash, elute with cysteine. Pass eluate through a small cation-exchange column (AG 50 W) (Bio-Rad) to remove cysteine, elute this column with 4 mL 10 mM HCl. (See Clin. Chim. Acta 1979, 95, 189.) Neutralize 250 μL eluate with 25 μL 100 mM NaOH, add 5 mL 50 mM pH 9.0 carbonate buffer containing 10 mM disodium EDTA, add 500 μL 0.5 mM N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in acetone, heat at 37° for 20 h, dilute 1:5 with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8

Mobile phase: MeOH:2 mM sodium phosphate 15:85, containing 10 mM tetramethylammonium hydroxide, pH adjusted to 7.4 with 6 M HCl

Flow rate: 0.75

Injection volume: 100

Detector: F ex 400 em 480

CHROMATOGRAM

Retention time: 20

Limit of detection: 53 fmole

OTHER SUBSTANCES

Extracted: mercaptoacetate

REFERENCE

Kågedal,B.; Källberg,M. Reversed-phase ion-pair high-performance liquid chromatography of mercaptoacetate and N-acetylcysteine after derivatization with N-(1-pyrene)maleimide and N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide, *J.Chromatogr.*, **1982**, *229*, 409–415.

SAMPLE

Matrix: urine

Sample preparation: 250 μL Urine + 10 μL 14.5 mg/mL dithioerythritol in water + 100 μL phosphate buffer. After 30 min remove a 250 μL aliquot and add it to 250 μL citrate buffer and 50 μL 1 mg/mL 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in MeOH, after 20 min inject a 20 μL aliquot. (Phosphate buffer was 17.8 g $Na_2HPO_4.2H_2O$ in 200 mL water, pH adjusted to 8.3 with NaOH. Citrate buffer was 29.4 g sodium citrate and 744 mg EDTA in 200 mL water, pH adjusted to 8.3 with NaOH.)

HPLC VARIABLES

Column: 300 × 4.6 7 μm Nucleosil C18

Mobile phase: MeCN:0.5% Na_2HPO_4 30:70

Column temperature: 30

Flow rate: 1.6

Injection volume: 20

Detector: UV 470

CHROMATOGRAM

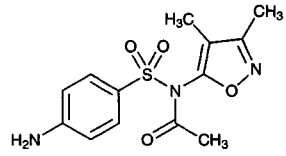
Retention time: 8

Limit of detection: 5 μM

KEY WORDS
derivatization

REFERENCE
Frank,H.; Thiel,D.; Langer,K. Determination of N-acetyl-L-cysteine in biological fluids, *J.Chromatogr.*, **1984**, *309*, 261–267.

Acetyl sulfisoxazole



Molecular formula: $C_{13}H_{15}N_3O_4S$
Molecular weight: 309.4
CAS Registry No.: 80-74-0
Merck Index: 9125

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 200 µL Plasma + 400 µL 0.2 µg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min. Remove the supernatant and evaporate it to 100 µL under a stream of nitrogen, inject a 50 µL aliquot. Urine. 100 µL Urine + 200 µL 12 µg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min, inject a 10 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 10 µm Lichrosorb RP-18 Hibar II
Mobile phase: MeOH:water:glacial acetic acid 32:68:0.06, pH adjusted to 4.7 with 4 M NaOH
Flow rate: 1.2
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM

Retention time: 9
Internal standard: N^4-acetylsulfamethoxazole (11.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: sulfisoxazole

KEY WORDS

plasma

REFERENCE

Jung,D.; Oie,S. "High-pressure" liquid chromatography of sulfisoxazole and N^4-acetylsulfisoxazole in body fluids, *Clin.Chem.*, **1980**, *26*, 51–54.

SAMPLE

Matrix: formulations
Sample preparation: Extract 1 mL Suspension with three 15 mL aliquots of chloroform, combine the organic layers and make up to 50 mL with chloroform, filter (0.45 µm silver membrane, Selas Corp.). Evaporate a 2 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 5 mL 330 µg/mL benzanilide in MeCN, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 µm µBondapak C18
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: benzanilide (11)

OTHER SUBSTANCES

Simultaneous: sulfanilamide, sulfanilic acid, sulfisoxazole
Noninterfering: erythromycin ethylsuccinate

KEY WORDS

oral suspensions; suspensions

REFERENCE

Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

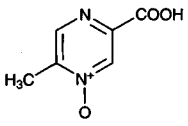
CHROMATOGRAM

Retention time: k' 2.86

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Acipimox



Molecular formula: $C_6H_6N_2O_3$
Molecular weight: 154.13
CAS Registry No.: 51037-30-0
Merck Index: 113

SAMPLE

Matrix: perfusate
Sample preparation: 1 mL Perfusate + 100 µL 20 µg/mL sulfanilamide + 1 mL 1 M phosphoric acid + 10 mL ethyl acetate:isopropanol 90:10, vortex for 3 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 150 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 4 µm Novapak phenyl in a Z-module radial compression module
Mobile phase: MeOH:buffer 4:96 (Buffer was 25 mM K_2HPO_4 + 5 mM tetrabutylammonium + 5 mM triethylamine, pH adjusted to 6.8 with concentrated phosphoric acid.)
Flow rate: 3
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Internal standard: sulfanilamide
Limit of quantitation: 100 ng/mL

REFERENCE

Ghabrial,H.; Czuba,M.A.; Stead,C.K.; Smallwood,R.A.; Morgan,D.J. Transfer of acipimox across the isolated perfused human placenta, *Placenta.*, **1991**, *12*, 653–661.

SAMPLE

Matrix: urine
Sample preparation: Dilute urine with water 1:10, inject a 20 µL aliquot directly.

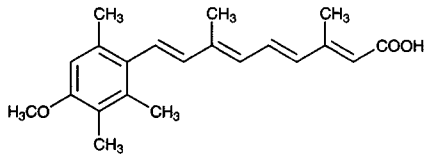
HPLC VARIABLES

Column: 250 × 3 Partisil PAC 10
Mobile phase: MeCN:pH 2.5 citrate-phosphate buffer 20:80
Flow rate: 1.4
Injection volume: 20
Detector: UV 269

REFERENCE

Musatti,L.; Maggi,E.; Moro,E.; Valzelli,G.; Tamassia,V. Bioavailability and pharmacokinetics in man of acipimox, a new antilipolytic and hypolipemic agent, *J.Int.Med.Res.*, **1981**, *9*, 381–386.

Acitretin



Molecular formula: $C_{21}H_{26}O_3$
Molecular weight: 326.44
CAS Registry No.: 55079-83-9
Merck Index: 114
Lednicer No.: 4 35

SAMPLE

Matrix: bile
Sample preparation: 20 µL Bile + 250 µL 1 M pH 5.5 acetate buffer + 20 µL Glusulase, shake at 37° for 4 h, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 Econosphere C18 (Alltech)
Mobile phase: MeOH:0.01% acetic acid 78:22
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 17.6

OTHER SUBSTANCES

Extracted: isoacitretin, Ro 23-4293, Ro 23-3571, metabolites

KEY WORDS

rat

REFERENCE

Cotler,S.; Chang,D.; Henderson,L.; Garland,W.; Town,C. The metabolism of acitretin and isoacitretin in the *in situ* isolated perfused rat liver, *Xenobiotica*, **1992**, *22*, 1229–1237.

SAMPLE

Matrix: bile, blood, perfusate, tissue
Sample preparation: Homogenize 1 g tissue and 4 mL ice-cold pH 7.4 Krebs-Henseleit buffer. Dilute bile with an equal volume of 200 mM pH 5 sodium acetate buffer. 100 µL Plasma, perfusate, diluted bile, or tissue homogenate + 20 µL MeCN + 350 µL MeCN:1-butanol 50:50 + 20 µL 37.6 µg/mL retinyl acetate, vortex for 1 min, add 300 µL 1 g/mL K_2HPO_4 in water, vortex for 30 s, centrifuge at 13600 g for 3 min, inject a 200 µL aliquot of the organic layer. (Hydrolyze conjugates in bile as follows. 100 µL Diluted bile + 8 µL 100000 U/mL β-glucuronidase (Helix pomatia, Sigma), heat at 37° for 5 h.)

HPLC VARIABLES

Guard column: 10 mm long Supelcosil LC-18 guard column
Column: 250 × 4.6 5 µm Supelcosil LC-18
Mobile phase: Gradient. A was MeCN:buffer 20:80. B was MeCN. A:B 65:35 for 10 min, 31:69 for 17 min (step gradient), re-equilibrate for 6 min. (Buffer was 0.8 g ammonium acetate and 10 mL glacial acetic acid in 200 mL water.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 200
Detector: UV 350

CHROMATOGRAM

Retention time: 15.7 (acitretin), 15.4 (cis-acitretin)

Internal standard: retinyl acetate (25.0)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES
Extracted: etretinate, metabolites

KEY WORDS
rat; liver; plasma; protect from light

REFERENCE
Decker,M.A.; Zimmerman,C.L. Simultaneous determination of etretinate, acitretin and their metabolites in perfusate, perfusate plasma, bile or hepatic tissue with reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *667*, 105–113.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 1.5 mL EtOH, freeze at -20° for 30 min, centrifuge. Inject a 1.4 mL aliquot of the supernatant onto column A and elute to waste with mobile phase A (time not given). Elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A C18-Corasil or C18-Lichrospher; B two 250 × 4 Supersphere 100 RP-18 end-capped columns in series
Mobile phase: A. MeCN:acetic acid (ratio not given) containing 1% ammonium acetate; B. Gradient. MeCN:acetic acid:10% ammonium acetate:water 60:1:6:30, 95:2:0.5:2, and 99:0.5:0:0.5 (times not given).
Detector: UV 360

CHROMATOGRAM
Internal standard: acitretin

OTHER SUBSTANCES
Extracted: isotretinoin, 4-oxo-isotretinoin, 4-oxo-tretinoin, tretinoin

KEY WORDS
plasma; column-switching; acitretin is IS

REFERENCE
Chen,C.; Mistry,G.; Jensen,B.; Heizmann,P.; Timm,U.; van Brummelen,P.; Rakhit,A.K. Pharmacokinetics of retinoids in women after meal consumption or vitamin A supplementation, *J.Clin.Pharmacol.*, **1996**, *36*, 799–808.

SAMPLE
Matrix: blood
Sample preparation: Mix 0.2-1 mL plasma and 100 μL buffer, extract with 2 mL diethyl ether:ethyl acetate 50:50 for 5 min. Centrifuge at 2000 g for 10 min at 4°, evaporate the organic phase to dryness. Dissolve the residue in 30-100 μL MeOH, inject an aliquot. (Solution was prepared in yellow amber glass and all handling was performed in a room with dim yellow light! Buffer was 25 mM KH_2PO_4 containing 40 mM Na_2HPO_4, pH 7.)

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:MeOH:THF 33.25:61.75:5. B was 2% acetic acid. A:B from 75:25 to 88:12 over 11 min, maintain at 88:12 for 19 min, return to initial condition at 30 min, equilibrate for 10 min.
Flow rate: 1

Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 21 (acitretin), 19.8 (13-cis-acitretin)
Internal standard: acitretin, 13-cis-acitretin

OTHER SUBSTANCES
Extracted: isotretinoin, tretinoin, 9-cis-retinoic acid
Noninterfering: acetaminophen, acyclovir, alprazolam, amikacin, amitriptyline, amphotericin B, aspirin, atenolol, bromazepam, caffeine, carbamazepine, ceftriaxone, chlorpromazine, cimetidine, clonazepam, dextromethorphan, diazepam, erythromycin, flunitrazepam, haloperidol, ketoconazole, lorazepam, meprobamate, metronidazole, methylprednisolone, miconazole, midazolam, nifedipine, nitrazepam, netilmicin, nordiazepam, nystatin, oxazepam, phenytoin, prednisolone, prednisone, sulconazole, theophylline, thiopental, zidovudine

KEY WORDS
plasma; rabbit; rat; acitretin is IS

REFERENCE
Disdier,B.; Bun,H.; Catalin,J.; Durand,A. Simultaneous determination of all-trans-, 13-cis, 9-cis-retinoic acid and their 4-oxometabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 143–154.

SAMPLE
Matrix: blood
Sample preparation: 0.5-2 mL Plasma + 100 μL pH 7 phosphate buffer + 2 mL diethyl ether:ethyl acetate 50:50, vortex gently for 5 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 30-100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:1% aqueous acetic acid 85:15
Flow rate: 1.5
Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 12
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: 13-cis-acitretin, tretinoin, etretinate, 4-oxo-13-cis-retinoic acid
Noninterfering: antidepressants, benzodiazepines, psoralen
Interfering: isotretinoin

KEY WORDS
plasma; handle under yellow light

REFERENCE
Bun,H.; al-Mallah,N.R.; Aubert,C.; Cano,J.P. High-performance liquid chromatography of aromatic retinoids and isotretinoin in biological fluids, *Methods Enzymol.*, **1990**, *189*, 167–172.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 10 μL 9.8 μg/mL retinyl palmitate in MeOH + 1.5 mL EtOH + 500 μL 2 M HCl, vortex for 30 s, add 5 mL water, vortex for 30 s, add 7.5 mL n-hexane, rotate for 15 min, centrifuge at 1500 g for 6 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm CP-Spher Si 5 μm (Chrompack)
Mobile phase: Dichloromethane:acetic acid 99.8:0.2 (?)
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 10 (13-cis), 11.5 (all-trans)
Internal standard: retinyl palmitate (6)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: etretinate

KEY WORDS
plasma; normal phase; pharmacokinetics; protect from light

REFERENCE
De Leenheer,A.P.; Lambert,W.E.; De Bersaques,J.P.; Kint,A.H. High-performance liquid chromatographic determination of etretinate and all-*trans*- and 13-*cis*-acitretin in human plasma, *J.Chromatogr.*, **1990**, *500*, 637–642.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 1 μg/mL retinoic acid in methyl acetate + 500 μL pH 7.4 phosphate buffer + 200 μL methyl acetate + 4 mL diethyl ether, rotate at 20 rpm for 10 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 150 μL aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm LiChrosorb Si 60
Mobile phase: Hexane:methyl benzoate:propionic acid 375:25:1
Flow rate: 2
Injection volume: 150
Detector: UV 365

CHROMATOGRAM
Retention time: 10.5
Internal standard: retinoic acid (5.2)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: etretinate, isoacitretin

KEY WORDS
plasma; rat; normal phase; pharmacokinetics

REFERENCE
McNamara,P.J.; Blouin,R.A. Pharmacokinetic profile of two aromatic retinoids (etretinate and acitretin) in the obese Zucker rat, *J.Pharm.Sci.*, **1990**, *79*, 301–304.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 1 mL 100 ng/mL Ro 12-7554 and 100 ng/mL isotretinoin in EtOH, vortex, stand at 4° for 15 min, centrifuge at 1800 g for 3 min, inject a 500 μL aliquot onto column A with mobile phase A and elute for 7 min, elute column A in backflush mode with mobile phase A for 3 min, backflush contents of column A onto column B with mobile phase B and start the gradient for mobile phase B. At the end of the process flush the lines with component B of mobile phase B, re-equilibrate columns for 4 min. (Keep sample at 20° in the autosampler.)

HPLC VARIABLES

Column: A 14 × 4.6 37-50 μm Bondapak C18 Corasil (column fitted with 3 μm sieves not glass fiber filters); B 30 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1

Mobile phase: A MeCN:1% ammonium acetate 10:90; B Gradient. A was MeCN:water:10% ammonium acetate:acetic acid 600:400:4:30. B was MeCN:water:10% ammonium acetate: acetic acid 850:146:4:10. A:B 100:0 to 0:100 over 8 min, stay at 0:100 for 11 min.

Flow rate: A 1.5; B 1

Injection volume: 500

Detector: UV 360

CHROMATOGRAM

Retention time: 16

Internal standard: Ro 12-7554 (ethyl all-trans-9-(2,6-dichloro-4-methoxy-m-tolyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate) (24) and isotretinoin (17)

Limit of detection: 0.5-1 ng/mL

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: etretinate, 13-cis-acitretin, metabolites

KEY WORDS

plasma; column-switching

REFERENCE

Wyss,R. Determination of retinoids in plasma by high-performance liquid chromatography and automated column switching, *Methods Enzymol.*, **1990**, *189*, 146–155.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 10 μL 10 μg/mL 13-demethylretinoic acid in MeOH + 1.5 mL EtOH, vortex for 30 s, add 500 μL 2 M HCl, add 5 mL water, vortex for 30 s, add 7.5 mL n-hexane, rotate for 15 min, centrifuge at 1500 g for 6 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot. (Perform all manipulations in dim light.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Chromspher

Mobile phase: n-Hexane:methyl salicylate:acetic acid 200:18:0.6

Flow rate: 0.85

Injection volume: 50

Detector: UV 360

CHROMATOGRAM

Retention time: 12 (13-cis), 13 (all-trans)

Internal standard: 13-demethylretinoic acid (10)
Limit of quantitation: 3-4 ng/mL

KEY WORDS
plasma; normal phase

REFERENCE
Meyer,E.; Lambert,W.E.; De Leenheer,A.P.; Bersaques,J.P.; Kint,A.H. Improved quantitation of 13-*cis*- and all-*trans*-acitretin in human plasma by normal-phase high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *570*, 149–156.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL MeOH, vortex, add 200 μL 100 mM pH 7.4 phosphate buffer, add 5 mL 2.5 ng/mL retinoic acid in diethyl ether, rotate vertically at 20 rpm for 30 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Rainin)
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:1%acetic acid 83:17
Flow rate: 1
Injection volume: 120
Detector: UV 365

CHROMATOGRAM
Internal standard: retinoic acid
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: isoacitretin, vitamin A

KEY WORDS
plasma; rat; handle under yellow light; pharmacokinetics

REFERENCE
Small,D.S.; McNamara,P.J. Acitretin elimination in Sprague-Dawley rats pretreated with phenobarbital or β-naphthoflavone, *Drug Metab.Dispos.*, **1995**, *23*, 465–472.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg methyl-C1 Accubond SPE cartridge (J&W) with three 1 mL portions of MeOH and three 1 mL portions of 1% ammonium acetate. 500 μL Plasma + 20 μL MeCN containing 10 mM BHT + 1 mL 10 mM BHT in isopropanol, vortex, rotate for 15 min, centrifuge at 16000 g for 10 min. Remove the supernatant and add it to 11 mL 1% ammonium acetate, add to the SPE cartridge, wash with 1 mL 0.1% ammonium acetate, wash with 1 mL MeOH:0.1% ammonium acetate 50:50, dry under vacuum for 30 s, elute with 1.5 mL 10 mM BHT in MeCN. Add 10 μL pentafluorobenzyl bromide and 10 μL 10 mg/mL potassium carbonate in MeCN:water 50:50 to the eluate, vortex, let stand at room temperature for 1 h, evaporate to dryness under reduced pressure for 2 h, reconstitute with 20-100 μL 10 mM BHT in MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18 + 75 × 3.9 Nova-Pak C18 (in series)

Mobile phase: Gradient. MeCN:buffer 80:20 for 10 min, to 90:10 (step gradient). (Buffer was 100 mM ammonium acetate adjusted to pH 5.0 with acetic acid.)
Column temperature: 40
Injection volume: 20
Detector: UV 369, MS Hewlett-Packard model 5988A, particle beam interface nebulizer 60°, helium 35 psi, m/z 325

CHROMATOGRAM
Retention time: 16
Internal standard: acitretin

OTHER SUBSTANCES
Extracted: isotretinoin (m/z 299), tretinoin (m/z 299), 9-cis-retinoic acid (m/z 299)

KEY WORDS
plasma; protect from light; derivatization; SPE; acitretin is IS

REFERENCE
Lehman,P.A.; Franz,T.J. A sensitive high-pressure liquid chromatography/particle beam/mass spectrometry assay for the determination of *all-trans*-retinoic acid and 13-*cis*-retinoic acid in human plasma, *J.Pharm.Sci.*, **1996**, *85*, 287–290.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 300-1000 μL Plasma + 100 μL pH 7 Titrisol buffer + IS in MeOH + 2 mL diethyl ether:ethyl acetate 1:1, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 30-50 μL MeOH, inject an aliquot. Tissue. Add IS, homogenize skin sample with 6 mL ethyl acetate:diethyl ether 1:1 with an 8 mm cutter at 24000 rpm below 21° (Ultra Turrax T25), centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen below 21°, reconstitute the residue in 30-50 μL, inject an aliquot. (Protect all samples from light.)

HPLC VARIABLES
Column: 250 × 4.2 5 μm Nucleosil C18
Mobile phase: MeOH:MeCN:1.5% acetic acid 59.5:25.5:15
Flow rate: 1.2
Detector: UV 350

CHROMATOGRAM
Retention time: 6 (13-cis), 6.9 (all-trans)
Internal standard: arotinoid methyl sulfone (Ro 15-1570) (7.8)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; skin

REFERENCE
Laugier,J.-P.; Surber,C.; Bun,H.; Geiger,J.-M.; Wilhelm,K.-P.; Durand,A.; Maibach,H.I. Determination of acitretin in the skin, in the suction blister, and in plasma of human volunteers after multiple oral dosing, *J.Pharm.Sci.*, **1994**, *83*, 623–628.

SAMPLE
Matrix: culture media
Sample preparation: 100 μL Culture media + 200 μL ice-cold EtOH, mix thoroughly, let stand for 15 min, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Whatman CO:PELL ODS guard column
Column: 100 × 8 5 μm Nova-Pak C18 (radial-packed)
Mobile phase: MeOH:100 mM pH 7.0 ammonium acetate 90:10
Flow rate: 1
Detector: UV 340

CHROMATOGRAM
Retention time: 7.00
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: isotretin, motretinid, all-trans-retinoic acid, Vitamin A (retinol), retinal, etretinate

REFERENCE
Kochhar,D.M.; Penner,J.D.; Minutella,L.M. Biotransformation of etretinate and developmental toxicity of etretin and other aromatic retinoids in teratogenesis bioassays, *Drug Metab.Dispos.*, **1989**, *17*, 618–624.

SAMPLE
Matrix: perfusate
Sample preparation: 500 μL Perfusate + 1 mL acetone + 20 μL 26 μg/mL retinyl acetate, vortex for 1 min, centrifuge at 4° at 1300 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: LC-18 pellicular (Supelco)
Column: 150 × 4.6 5 μm Supelcosil C18
Mobile phase: MeCN:water 84:16 containing 0.8 g/L ammonium acetate and 10 mL/L glacial acetic acid
Flow rate: 1.5
Detector: UV 350

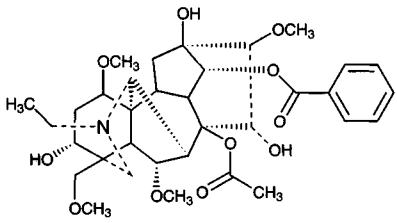
CHROMATOGRAM
Internal standard: retinyl acetate

OTHER SUBSTANCES
Extracted: etretinate

KEY WORDS
do not use PTFE or plastic; rat

REFERENCE
Pithavala,Y.K.; Odishaw,J.L.; Han,S.; Wiedmann,T.S.; Zimmerman,C.L. Retinoid absorption from simple and mixed micelles in the rat intestine, *J.Pharm.Sci.*, **1995**, *84*, 1360–1365.

Aconitine



Molecular formula: $C_{34}H_{47}NO_{11}$
Molecular weight: 645.75
CAS Registry No.: 302-27-2
Merck Index: 120

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 233

CHROMATOGRAM

Retention time: 5.95

Limit of detection: <120 ng/mL

KEY WORDS

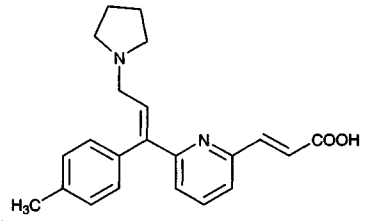
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Acrivastine



Molecular formula: $C_{22}H_{24}N_2O_2$
Molecular weight: 348.44
CAS Registry No.: 87848-99-5
Merck Index: 129
Lednicer No.: 4 105

SAMPLE

Matrix: urine

Sample preparation: Condition a C18 SepPak SPE cartridge with 5 mL MeOH and 10 mL water. Urine. Centrifuge, add 0.1-7 mL to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen in a warm water bath, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject an aliquot. Feces. Lyophilize, grind to powder, weigh out 0.22-1.28 g, add 10 mL MeOH:water 80:20, vortex vigorously, sonicate using a microtip probe for 15 s, shake vigorously for 30 min, centrifuge, remove supernatant, repeat extraction (without sonication) twice. Combine extracts and dilute them with water to 20% MeOH concentration. Add to two SPE cartridges connected in series, wash with 3 mL water, elute with 4 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen in a warm water bath, reconstitute the residue in 300 μL MeOH, filter (0.45 μm), inject an aliquot. Plasma. 700 μL Plasma + 700 μL water, add to SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm C8 Spherisorb Octyl
Column: 150 × 4 5 μm Econosphere C8
Mobile phase: Gradient A. A was MeCN:100 mM ammonium acetate 5:95. B was MeCN: 100 mM ammonium acetate 50:50. A:B from 100:0 to 80:20 over 10 min then to 0:100 over 10 min, maintain at 0:100 for 5 min.
Flow rate: 0.9
Detector: UV 235

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Extracted: metabolites

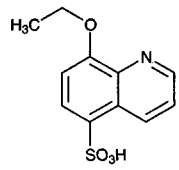
KEY WORDS

SPE; pharmacokinetics

REFERENCE

McNulty,M.J.; Deal,D.L.; Nelson,F.R.; Weller,S.; Chandrasurin,P.; Shockcor,J.; Findlay,J.W. Disposition of acrivastine in the male beagle dog, *Drug Metab.Dispos.*, **1992**, *20*, 679–687.

Actinoquinol



Molecular formula: $C_{11}H_{11}NO_4S$
Molecular weight: 253.28
CAS Registry No.: 15301-40-3, 7246-07-3 (sodium salt)
Merck Index: 143

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM

Retention time: 4.637

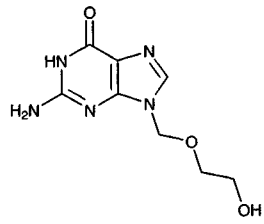
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acyclovir



Molecular formula: $C_8H_{11}N_5O_3$
Molecular weight: 225.21
CAS Registry No.: 59277-89-3, 69657-51-8 (sodium salt)
Merck Index: 148
Lednicer No.: 3 229; 4 31; 4 116; 4 165

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 35% perchloric acid, mix, centrifuge at 4° at 1500 g for 15 min. Inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM pH 3.5 KH_2PO_4
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 9.8
Limit of detection: 2 ng
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: azathioprine, bumetamide, cyclosporine, cimetidine, dexamethasone, furosemide, ganciclovir, guanine, guanosine, hypoxanthine, 6-mercaptopurine, phenobarbital, prednisone, pristinamycine, pyostacine, ranitidine, salicylic acid, uric acid, vancomycine, xanthine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Boulieu,R.; Gallant,C.; Silberstein,N. Determination of acyclovir in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 233–236.

SAMPLE

Matrix: blood
Sample preparation: 250 μL Plasma + 30 μL 60-62% perchloric acid, vortex for 30 s, centrifuge at 12800 g for 25 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 30-40 μm Perisorb RP-18 (Upchurch Scientific)
Column: 250 × 4 7 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 1:99 (Buffer was 20 mM NaH_2PO_4adjusted to pH 2.5 with 60–62% perchloric acid.)
Flow rate: 1.2
Injection volume: 50
Detector: F ex 270 em 380

CHROMATOGRAM

Retention time: 9.59

Limit of detection: 30 ng/mL
Limit of quantitation: 62.5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Peh,K.K.; Yuen,K.H. Simple high-performance liquid chromatographic method for the determination of acyclovir in human plasma using fluorescence detection, *J.Chromatogr.B*, **1997**, *693*, 241–244.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL MeCN to 500 μL plasma, vortex briefly, centrifuge at 2000 g for 3 min, add 2 mL chloroform (Caution! Chloroform is a carcinogen!) to the supernatant, vortex. Remove the aqueous supernatant layer, remove traces of the organic solvent under a stream of nitrogen at 80° for 3 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrocart RP8
Mobile phase: MeCN:10 mM pH 5 ammonium acetate buffer 2:98
Flow rate: 1
Injection volume: 30
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: acyclovir (7)

OTHER SUBSTANCES
Extracted: gangiclovir

KEY WORDS
plasma; acyclovir is IS

REFERENCE
Cociglio,M.; Peyrière,H.; Hillaire-Buys,D.; Alric,R. Application of a standardized coextractive cleanup procedure to routine high-performance liquid chromatography assays of teicoplanin and ganciclovir in plasma, *J.Chromatogr.B*, **1998**, *705*, 79–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak Light C18 cartridge (Waters) with 1 mL MeOH and 1 mL water. Mix 500 μL serum with 500 μL 50% saturated NaCl in water or 100 μL urine with 900 μL 50% saturated NaCl in water. Push the mixture through the cartridge with a plastic syringe at a flow-rate 25 μL/s in this and subsequent steps, wash with 500 μL 50% saturated NaCl in water and elute with 1 mL 3% MeCN in 38 mM phosphoric acid. Collect the last 750 μL aliquot of the eluate and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:30 mM pH 2.1 phosphate buffer containing 5 mM dodecyl sulfate 18:82 (Prepare mobile phase as follows. Dissolve 4.08 g potassium dihydrogen phosphate, 1.45 g sodium dodecyl sulfate and 15 mL 3.85 mM phosphoric acid in 800 mL water, add 180 mL MeCN an make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 285 em 380

CHROMATOGRAM
Retention time: 1.70
Limit of detection: 120 nM (plasma), 600 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: guanosine
Noninterfering: azathioprine, cyclosporine, furosemide, nifedipine, prednisolone, sulfamethoxazole, trimethoprim

KEY WORDS
SPE; serum; pharmacokinetics

REFERENCE
Svensson,J.-O.; Barkholt,L.; Säwe,J. Determination of acyclovir and its metabolite 9-carboxymethoxymethylguanine in serum and urine using solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *690*, 363–366.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.073

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 10 μm CLC-ODS (Shimadzu)

Mobile phase: MeOH:2% acetic acid 10:90
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13
Limit of detection: 1.22 μg/mL

OTHER SUBSTANCES
Simultaneous: guanine

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Zhang,S.S.; Liu,H.X.; Chen,Y.; Yuan,Z.B. Comparison of high performance capillary electrophoresis and liquid chromatography for the determination of acyclovir and guanine in pharmaceuticals and urine, *Biomed.Chromatogr.*, **1996**, *10*, 256–257.

SAMPLE
Matrix: tissue
Sample preparation: Extract skin (20 μm in thickness and 1.2 μL in volume) with 200 μL distilled water at 60° for 15 min, vortex twice for 10 s during extraction, cool, add 200 μL 148 mM perchloric acid, centrifuge at 5000 g for 10 min, filter (0.45 μm nylon, Lida, USA), inject an aliquot.

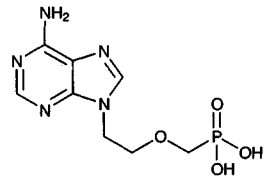
HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac C18
Mobile phase: Water
Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 8 ng/mL

KEY WORDS
skin

REFERENCE
Volpato,N.M.; Santi,P.; Laureri,C.; Colombo,P. Assay of acyclovir in human skin layers by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 515–520.

Adefovir



Molecular formula: $C_8H_{12}N_5O_4P$
Molecular weight: 273.18
CAS Registry No.: 106941-25-7

SAMPLE

Matrix: blood
Sample preparation: Mix 100μL plasma with 200 μL 0.1% trifluoroacetic acid in MeCN. Evaporate to dryness under reduced pressure. Reconstitute dry sample in 200 μL derivatization solution, vortex, centrifuge. Remove the supernatant, incubate it at 95° for 40 min. Evaporate derivatized sample to dryness, reconstitute in 100 μL mobile phase A without MeCN. Inject a 50 μL aliquot. (Derivatization solution was 0.34% chloroacetaldehyde in 100 mM pH 4.5 sodium acetate.)

HPLC VARIABLES

Guard column: 15 × 3.2 Brownlee RP-18 Newguard
Column: 150 × 4.6 Zorbax RX-C18
Mobile phase: Gradient. A was MeCN:25 mM pH 6 potassium phosphate buffer containing 5 mM tetrabutyl ammonium hydrogen phosphate 2:98. B was MeCN:25 mM pH 6 potassium phosphate buffer containing 5 mM tetrabutyl ammonium hydrogen phosphate 65:45. A:B 100:0 for 2 min, to 0:100 over 13 min, return to 100:0 immediately.
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: F ex 236 em 420

KEY WORDS

plasma; derivatization; pharmacokinetics; dog

REFERENCE

Cundy,K.C.; Sue,I-L.; Visor,G.C.; Marshburn,J.; Nakamura,C.; Lee,W.A.; Shaw,J.-P. Oral formulations of adefovir dipivoxil: In vitro dissolution and in vivo bioavailability in dogs, *J.Pharm.Sci.*, **1997**, *86*, 1334–1338.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma, serum. Add trichloroacetic acid to 100 μL serum or plasma so that the final concentration of trichloroacetic acid is 100 mg/mL, shake vigorously for 10 min, centrifuge at 9000 g for 5 min, add the supernatant to an equal volume of tri-n-octylamine:Freon 20:80, shake vigorously for 30 min, discard the lower organic layer. Treat the aqueous layer with pH 4.7 ammonium acetate buffer (final concentration 160 mM) and chloroacetaldehyde (final concentration 40 mM), heat at 95° for 40 min, cool at 4°, inject an aliquot equivalent to 60 μL plasma or serum. Urine. Centrifuge urine, treat with pH 4.7 ammonium acetate buffer and chloroacetaldehyde, heat at 95° for 20 min (Antimicrob. Agents Chemother. 1996, 40, 22).

HPLC VARIABLES

Column: 125 × 4.6 4 μm Superspher 60 C8 (Merck)
Mobile phase: Gradient. A was MeCN:2.5 mM pH 5.0 $(NH_4)H_2PO_4$ containing 2 mM tetrabutylammonium hydrogen sulfate 5:95. B was MeCN:75 mM pH 5.0 $(NH_4)H_2PO_4$ containing 2 mM tetrabutylammonium hydrogen sulfate 15:85. A:B 100:0 for 4 min, to 0:100 over 2 min, maintain at 0:100 for 4 min, re-equilibrate at initial conditions for 8 min.
Flow rate: 1
Detector: F ex 254 (filter) em 425 (filter)

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CHROMATOGRAM

Retention time: 12.0
Limit of quantitation: 250 nM

OTHER SUBSTANCES

Extracted: adenosine monophosphate, (R,S)-9-(3-fluoro-2-phosphonylmethoxypropyl) adenine (FPMPA), (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA)

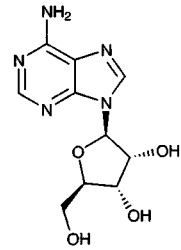
KEY WORDS

monkey; cat; human; plasma; serum; derivatization

REFERENCE

Naesens,L.; Balzarini,J.; De Clercq,E. Acyclic adenine nucleoside phosphonates in plasma determined by high-performance liquid chromatography with fluorescence detection, *Clin.Chem.*, **1992**, *38*, 480–485.

Adenosine



Molecular formula: $C_{10}H_{13}N_5O_4$
Molecular weight: 267.24
CAS Registry No.: 58-61-7
Merck Index: 152

SAMPLE
Matrix: blood
Sample preparation: Plasma, ultrafilter (Amicon) while centrifuging at 1000 g at 20° for 45 min, inject a 50 µL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: 10 × 3 5 µm Chromsep C18
Column: 100 × 4.6 3 µm Microspher C18
Mobile phase: MeCN:50 mM pH 3.7 Na_2HPO_4 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 3.7 nM

OTHER SUBSTANCES
Extracted: inosine

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Huszár,.; Bart,E.; Kollai,M. Isocratic high-performance liquid chromatographic determination of plasma adenosine, *Chromatographia*, **1996**, *42*, 318–322.

SAMPLE
Matrix: blood
Sample preparation: Add 4 mL cold 600 mM perchloric acid to 2 mL blood, let stand at 4° for 10 min, centrifuge. Neutralize a 3 mL aliquot of the supernatant by adding 300 µL 2.5 M potassium carbonate dropwise while stirring, centrifuge. Mix 200 µL of the supernatant with 5′-nucleotidase (from *Crotatus atrox* venom, activity 67 units/mg protein), keep at room temperature, inject 25 µL aliquot.

HPLC VARIABLES
Column: 100 × 8 5 or 10 µm Radial-Pak C18 Resolve cartridge (uncapped) (Waters)
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer:triethylamine 3.5:94.5:2, adjusted to pH 6.9 with phosphoric acid.
Column temperature: 24-26
Flow rate: 2.0
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 10.22
Limit of detection: 10-50 pmole

OTHER SUBSTANCES
Extracted: ATP, ADP, AMP

KEY WORDS
whole blood

REFERENCE
Nishikawa,T.; Suzuki,S.; Ohtani,H.; Shirai,M.; Nomyiama,S.; Kubo,H. Isocratic separation of adenosine 5′-triphosphate and its metabolites by reversed-phase high performance liquid chromatography: end-capped *versus* uncapped packings, *Anal.Sci.*, **1991**, *7*, 241–246.

SAMPLE
Matrix: blood
Sample preparation: Inject 10 μL plasma onto column A and column B in series with mobile phase A, elute with mobile phase A for 4 min then elute the contents of column B onto column C with mobile phase B, monitor the effluent from column C.

HPLC VARIABLES
Column: A 30 × 4.6 44-88 μm Butyl-Toyopearl 650-M (Tosoh); B 30 × 4.6 5 μm Develosil ODS-5 (Nomura); C 250 × 7.6 5 μm Asahipak GS-320H
Mobile phase: A MeCN:2 mM pH 7.4 phosphate buffer 5:95; B MeCN:50 mM bromoacetaldehyde + 150 mM NaCl 15:85, containing 25 mM citrate, pH 5.0
Column temperature: 40° (column C)
Flow rate: A 0.3; B 0.5
Injection volume: 10
Detector: F ex 254 em 400 following post-column heating at 115° in a 15 m × 0.25 mm i.d. reaction coil (Jasco RU-150F unit)

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: adenine
Noninterfering: adenosine triphosphate, adenosine monophosphate, cyclic adenosine monophosphate, adenosine diphosphate

KEY WORDS
plasma; hamster; human; column-switching; post-column reaction; derivatization

REFERENCE
Fujimori,H.; Sasaki,T.; Hibi,K.; Senda,M.; Yoshioka,M. Direct injection of blood samples into a high-performance liquid chromatographic adenine analyser to measure adenine, adenosine, and the adenine nucleotides with fluorescence detection, *J.Chromatogr.*, **1990**, *515*, 363–373.

SAMPLE
Matrix: blood
Sample preparation: 360 μL Blood + 40 μL stopping solution, centrifuge at 14000 g for 1 min. 100 μL Plasma + 10 μL 50% trichloroacetic acid, centrifuge for 5 min. Remove 75 μL of the supernatant and add it to 10 μL 2.3 M KOH, add 50 μL 1 M zinc sulfate, add 100 μL saturated barium hydroxide, vortex for 10 s, centrifuge at 14000 g for 5 min. Remove 100 μL supernatant (pH 5.4) and add it to 10 μL chloroacetaldehyde (45% in water (Fluka)), heat at 80° for 1 h, inject an aliquot. (Stopping solution was 1 mM dialazep, 10μM erythro-9-(2-hydroxy-3-nonyl)adenine, 2 μg/mL indomethacin (final concentrations).)

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was MeOH:10 mM pH 3.5 KH_2PO_4 12:88. B was MeOH:10 mM pH 3.5 KH_2PO_4 50:50. A for 8 min then B for 8 min, re-equilibrate for 4 min.
Flow rate: 1.5
Detector: F ex 280 em 380

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 0.2 pmole

KEY WORDS
plasma; cat; rat; dog; mouse; rabbit; guinea pig; derivatization

REFERENCE
Zhang,Y.; Geiger,J.D.; Lautt,W.W. Improved high-pressure liquid chromatographic-fluorometric assay for measurement of adenosine in plasma, *Am.J.Physiol.*, **1991**, *260*, G658–G664.

SAMPLE
Matrix: blood
Sample preparation: 70 μL Serum + 10 μL 1 mM D-glucosamine.HCl + 20 μL 1 M K_2HPO_4 + 10 μL benzoyl chloride + 25 μL 8 M NaOH, vortex at 2500 vibrations/min for 5 min, add 10 μL 1.4 M phosphoric acid and 100 μL ethyl acetate, vortex at 2500 vibrations/min for 1 min. Remove 25 μL of the ethyl acetate phase and add it to 100 μL MeCN:water 70:30, inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Kromasil 100 C18
Column: 250 × 4 5 μm Kromasil 100 C18
Mobile phase: Gradient. MeCN:water from 70:30 to 95:5 over 30 min.
Flow rate: 1
Injection volume: 50
Detector: UV 228 or MS, electrospray, Finnigan MAT, TSQ 700, flow rate 1 μL/min, 2.8 kV, drying gas 140

CHROMATOGRAM
Retention time: 10.9
Internal standard: D-glucosamine (9.7)
Limit of detection: 1-5 pmol

OTHER SUBSTANCES
Extracted: benzyl alcohol, dextrose, mannitol, 2-desoxy-D-glucose, cytidine, myoinositol, sucrose

KEY WORDS
serum; derivatization; fetal bovine serum

REFERENCE
Oehlke,J.; Brudel,M.; Blasig,I.E. Benzoylation of sugars, polyols and amino acids in biological fluids for high-performance liquid chromatographic analysis, *J.Chromatogr.B*, **1994**, *655*, 105–111.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wave-

length for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 2.697

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: dialysate

HPLC VARIABLES
Column: 80 × 4.6 C18 (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 7 KH_2PO_4 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Simultaneous: fludarabine, araA, 2-chloro-2'-arabinofluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine, 2-chloroadenosine, 5'-chloro-5'-deoxyadenosine, 2'-deoxyadenosine

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Structure-activity relationships of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine and related analogues: Protein binding, lipophilicity, and retention in reversed-phase LC, *J.Liq.Chromatogr.*, **1995**, *18*, 1123–1135.

SAMPLE
Matrix: perfusate
Sample preparation: Mix 700 μL perfusate (Earle's medium) with 750 μL 24 mM phosphoric acid to adjust pH to 5.4, add 10 μL chloroacetaldehyde, heat at 100° for 40 min, cool in ice, inject a 50 μL aliquot. (Earle's medium contains 116 mM NaCl, 22.6 mM sodium bicarbonate, 5.4 mM KCl, 1.8 mM calcium chloride, 0.8 mM magnesium sulfate, 1.0 mM NaH_2PO_4, 5.5 mM glucose, and 40 g/L dextran (MW 32000-48000), pH 8.5-8.6. Prepare chloroacetaldehyde by refluxing chloroacetaldehyde dimethyl acetal:1.5 M sulfuric acid 5:1 for 30 min, distil, collect the fraction boiling at 85-95°, use undiluted.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm NewGuard RP-18
Column: 100 × 4.6 3 μm ODS-Hypersil C18
Mobile phase: Gradient. MeOH:10 mM pH 6.7 $(NH_4)H_2PO_4$ from 0:100 to 1:99 over 1 min, to 3:97 over 1 min, to 6:94 over 1 min, to 10:90 over 1 min, to 15:85 over 1 min, to 23:77 over 1 min, to 35:65 over 1 min, to 50:50 over 1 min, to 60:40 over 1 min, to 80:20 over 0.5 min, maintain at 80:20 over 0.5 min, return to initial conditions over 0.2 min, re-equilibrate for 8.8 min.
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 275 em 415

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 2 nM

KEY WORDS
derivatization

REFERENCE
Slegel,P.; Kitagawa,H.; Maguire,M.H. Determination of adenosine in fetal perfusates of human placental cotyledons using fluorescence derivatization and reversed-phase high-performance liquid chromatography, *Anal.Biochem.*, **1988**, *171*, 124–134.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate (Krebs solution) + 40 μL chloroacetaldehyde + 360 μL buffer + 100 μL 600 nM vidarabine in water, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0° (Anal. Biochem. 1984, 137, 93).)

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Ultron N-phenyl (Shinwa, Kyoto)
Column: 150 × 4.6 5 μm Ultron N-phenyl (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 1.5:98.5, adjusted to pH 4.5 with 2-diethylaminoethanol (Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0.)
Flow rate: 1
Detector: F ex 305 em 420

CHROMATOGRAM
Retention time: 22
Internal standard: vidarabine (17)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: adenosine diphosphate, adenosine monophosphate, adenosine triphosphate

KEY WORDS
derivatization

REFERENCE

Mohri,K.; Takeuchi,K.; Shinozuka,K.; Bjur,R.A.; Westfall,D.P. Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery, *Anal.Biochem.*, **1993**, *210*, 262–267.

SAMPLE

Matrix: perfusate
Sample preparation: Direct injection of a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 octyl Spherisorb S5-ODS
Mobile phase: MeCN:100 mM KH_2PO_4 13:87
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 262

REFERENCE

Michael-Baruch,E.; Shiri,Y.; Cohen,S. Alkali halide-assisted penetration of neostigmine across excised human skin: A combination of structured water disruption and a Donnan-like effect, *J.Pharm.Sci.*, **1994**, *83*, 1071–1076.

SAMPLE

Matrix: perfusate, tissue
Sample preparation: Homogenize (glass to glass) tissue with 1 mL ice-cold 400 mM perchloric acid, centrifuge at 10000 g for 10 min, dilute the supernatant 10-fold with Krebs solution. Mix 1 mL (?) perfusate (Krebs solution) or tissue homogenate with 25 (perfusate) or 50 (tissue) μL chloroacetaldehyde, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0°.)

HPLC VARIABLES

Column: 5 μm Radial-Pak C18
Mobile phase: Gradient. A was 100 mM pH 6.0 phosphate buffer. B was MeOH:100 mM pH 6.0 phosphate buffer 25:75. A:B from 100:0 to 0:100 over 15 min (Waters concave curve 8), maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 2
Detector: F ex 300 em 420

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.5-1 pmole

OTHER SUBSTANCES

Extracted: adenosine diphosphate, adenosine monophosphate, adenosine triphosphate

KEY WORDS

derivatization; guinea pig; vas deferens

REFERENCE

Levitt,B.; Head,R.J.; Westfall,D.P. High-performance liquid chromatographic-fluorometric detection of adenosine and adenine nucleotides: Application to endogenous content and electrically induced release of adenyl purines in guinea pig vas deferens, *Anal.Biochem.*, **1984**, *137*, 93–100.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS2
Mobile phase: MeOH:200 mM pH 6.0 KH_2PO_4 3:97
Flow rate: 1.5
Detector: UV 260

OTHER SUBSTANCES
Simultaneous: ADP, AMP, ATP

REFERENCE
Ziganshin,A.U.; Ziganshina,L.E.; King,B.F.; Pintor,J.; Burnstock,G. Effects of P2-purinoceptor antagonists on degradation of adenine nucleotides by ecto-mucleotidases in folliculated oocytes of Xenopus laevis, *Biochem.Pharmacol.*, **1996**, *51*, 897–901.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 5 μm Supelcosil LC18 DB
Mobile phase: MeCN:10 mM ammonium acetate 4:96
Flow rate: 4
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 0.4

OTHER SUBSTANCES
Simultaneous: deoxyinosine, didanosine, dideoxyadenosine, hypoxanthine, inosine

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC18 DB
Mobile phase: MeCN:10 mM ammonium acetate 4:96
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: deoxyinosine, didanosine, dideoxyadenosine, hypoxanthine, inosine

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

SAMPLE

Matrix: tissue

Sample preparation: Deproteinize heart tissue with ice-cold 600 mM perchloric acid, neutralize, centrifuge at 4° at 26890 g for 15 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 3 μm LC-18-T (Supelco)

Column: 150 × 4.6 3 μm LC-18-T (Supelco)

Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 1:99, pH 7.0. B was MeOH:100 mM KH_2PO_4 containing 2.8 mM tetrabutylammonium hydroxide 30:70, pH 5.5. A:B 100:0 for 12 min, to 60:40 over 2 min, to 56:44 over 11 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 1.2

Injection volume: 20

Detector: UV 266

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Extracted: adenosine triphosphate, ascorbic acid

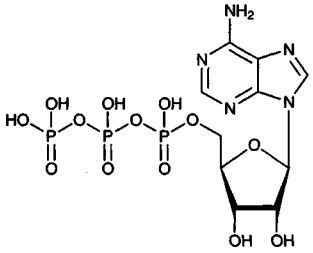
KEY WORDS

rat; heart

REFERENCE

Lazzarino,G.; Di Pierro,D.; Tavazzi,B.; Cerroni,L.; Giardina,B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography, *Anal.Biochem.*, **1991**, *197*, 191–196.

Adenosine triphosphate



Molecular formula: $C_{10}H_{16}N_5O_{13}P_3$
Molecular weight: 507.18
CAS Registry No.: 56-65-5
Merck Index: 154

SAMPLE

Matrix: blood

Sample preparation: Isolate mononuclear cells from 10 mL blood by a standard step-gradient density centrifugation procedure. Wash once with PBS, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash the pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, neutralize with 10 M KOH, bring to pH 7 with 1 M KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject a 50-2000 μL aliquot of the supernatant. (PBS was 8.1 g NaCl, 0.22 g KCl, 1.14 g $NaHPO_4$ (sic), 0.27 g KH_2PO_4 in 1 L water, pH 7.4.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil 10 SAX

Mobile phase: Gradient. A was 5 mM pH 2.8 $(NH_4)H_2PO_4$. B was 750 mM pH 3.5 $(NH_4)H_2PO_4$. A:B from 70:30 to 0:100 over 30 min (concave gradient, Waters no. 9). (At the start of each day pump through 20 mL 2 M $(NH_4)H_2PO_4$ then inject 100 μL 100 mM disodium EDTA into the initial mobile phase.)

Flow rate: 3

Injection volume: 50-2000

Detector: UV 262

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: ara-CTP (cytarabine triphosphate), Fara-ATP (fludarabine triphosphate), CTP, UTP, GTP

KEY WORDS

mononuclear cells

REFERENCE

Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

SAMPLE

Matrix: blood

Sample preparation: Dilute whole blood 10-100 fold with 320 mM sucrose, inject 10 μL of the diluted solution onto column A and column B in series with mobile phase A, elute with mobile phase A for 4 min then elute the contents of column B onto column C with mobile phase B, monitor the effluent from column C.

HPLC VARIABLES

Column: A 30 × 4.6 44-88 μm Butyl-Toyopearl 650-M (Tosoh); B 10 × 4 3 μm Hitachi gel 3013-N; C 50 × 4.6 3 μm Hitachi gel 3013-N

Mobile phase: A MeCN:water 15:85; B MeCN:50 mM bromoacetaldehyde + 150 mM NaCl 15:85, containing 25 mM citrate, pH 4.0
Column temperature: 45° (column C)
Flow rate: A 0.3; B 0.3
Injection volume: 10
Detector: F ex 254 em 400 following post-column heating at 115° in a 15 m × 0.25 mm i.d. reaction coil (Jasco RU-150F unit)

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: adenosine monophosphate, cyclic adenosine monophosphate, adenosine diphosphate
Noninterfering: adenine, adenosine

KEY WORDS
whole blood; hamster; rat; human; column-switching; post-column reaction; derivatization

REFERENCE
Fujimori,H.; Sasaki,T.; Hibi,K.; Senda,M.; Yoshioka,M. Direct injection of blood samples into a high-performance liquid chromatographic adenine analyser to measure adenine, adenosine, and the adenine nucleotides with fluorescence detection, *J.Chromatogr.*, **1990**, *515*, 363–373.

SAMPLE
Matrix: cell cultures
Sample preparation: Freeze in liquid nitrogen. 2 mL Frozen cell suspension + 1 mL cold 3 M perchloric acid, sonicate at 0° for 30 s, neutralize with 2 M KOH containing 300 mM 4-morpholinepropanesulfonic acid (MOPS), centrifuge at 3000 g for 10 min, inject an aliquot.

HPLC VARIABLES
Guard column: Valco guard column containing C18 pellicular material
Column: 150 × 4.6 Excellopak ODS C18 (R.E. Gourley)
Mobile phase: MeOH:100 mM pH 6.0 potassium phosphate 3.75:96.25
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Extracted: NADP+, NAD+, adenosine diphosphate, adenosine monophosphate

KEY WORDS
hepatocyte

REFERENCE
Litt,M.R.; Potter,J.J.; Mezey,E.; Mitchell,M.C. Analysis of pyridine dinucleotides in cultured rat hepatocytes by high-performance liquid chromatography, *Anal.Biochem.*, **1989**, *179*, 34–36.

SAMPLE
Matrix: cell suspensions
Sample preparation: 1 mL Cell suspension + 500 μL ice-cold MeCN + 500 μL water, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES
Column: Partisil-10 SAX
Mobile phase: Gradient. A was 30 mM pH 4.8 ammonium phosphate. B was MeCN:700 mM pH 4.6 ammonium phosphate 10:90. A:B 100:0 for 5 min then a convex gradient to 75:25 over 10 min then a convex gradient to 0:100 over 15 min, stay at 0:100 for 15 min
Flow rate: 1.7
Detector: UV 254

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Extracted: metabolites, didanosine

REFERENCE
Mukherji,E.; Au,J.L.-S.; Mathes,L.E. Differential antiviral activities and intracellular metabolism of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine in human cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1573–1579.

SAMPLE
Matrix: cells
Sample preparation: Separate cells from 10 mL whole blood, wash with phosphate-buffered saline, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, adjust pH to 7.0 with 10 KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil-10 SAX
Mobile phase: Gradient. 5 mM pH 2.8 $(NH_4)H_2PO_4$:750 mM pH 3.5 $(NH_4)H_2PO_4$ from 70:30 to 0:100 over 30 min (concave gradient Waters curve 9) (At the start of each day purge with 20 mL 2 M $(NH_4)H_2PO_4$, inject 100 μL 100 mM disodium EDTA under initial gradient conditions.)
Flow rate: 3
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: CTP, UTP, GTP, cytarabine triphosphate, fludarabine triphosphate

KEY WORDS
human cells

REFERENCE
Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 800 μL chloroform:MeOH 2:1, agitate vigorously for 1 min, centrifuge, inject a 5 μL aliquot of the upper water/MeOH layer.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 55:45 containing 9 mM KH_2PO_4, pH 5.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Extracted: ciprofibrate, metabolites

KEY WORDS
rat; liver

REFERENCE
Bronfman,M.; Amigo,L.; Morales,M.N. Activation of hypolipidaemic drugs to acyl-coenzyme A thioesters, *Biochem.J.*, **1986**, *239*, 781–784.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate (Krebs solution) + 40 μL chloroacetaldehyde + 360 μL buffer + 100 μL 600 nM vidarabine in water, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0° (Anal. Biochem. 1984, 137, 93).)

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Ultron N-phenyl (Shinwa, Kyoto)
Column: 150 × 4.6 5 μm Ultron N-phenyl (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 1.5:98.5, adjusted to pH 4.5 with 2-diethylaminoethanol (Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0.)
Flow rate: 1
Detector: F ex 305 em 420

CHROMATOGRAM
Retention time: 3.5
Internal standard: vidarabine (17)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: adenosine, adenosine diphosphate, adenosine monophosphate

KEY WORDS
derivatization

REFERENCE
Mohri,K.; Takeuchi,K.; Shinozuka,K.; Bjur,R.A.; Westfall,D.P. Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery, *Anal.Biochem.*, **1993**, *210*, 262–267.

SAMPLE

Matrix: perfusate, tissue

Sample preparation: Homogenize (glass to glass) tissue with 1 mL ice-cold 400 mM perchloric acid, centrifuge at 10000 g for 10 min, dilute the supernatant 10-fold with Krebs solution. Mix 1 mL (?) perfusate (Krebs solution) or tissue homogenate with 25 (perfusate) or 50 (tissue) μL chloroacetaldehyde, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0°.)

HPLC VARIABLES

Column: 5 μm Radial-Pak C18

Mobile phase: Gradient. A was 100 mM pH 6.0 phosphate buffer. B was MeOH:100 mM pH 6.0 phosphate buffer 25:75. A:B from 100:0 to 0:100 over 15 min (Waters concave curve 8), maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 2

Detector: F ex 300 em 420

CHROMATOGRAM

Retention time: 12

Limit of detection: 1 pmole

OTHER SUBSTANCES

Extracted: adenosine, adenosine diphosphate, adenosine monophosphate

KEY WORDS

derivatization; guinea pig; vas deferens

REFERENCE

Levitt,B.; Head,R.J.; Westfall,D.P. High-performance liquid chromatographic-fluorometric detection of adenosine and adenine nucleotides: Application to endogenous content and electrically induced release of adenyl purines in guinea pig vas deferens, *Anal.Biochem.*, **1984**, *137*, 93–100.

SAMPLE

Matrix: solutions

Sample preparation: 10 μL Solution + 200 μL buffer + 10 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in buffer + 40 μL 50 mM dansylethylenediamine in DMSO, mix, let stand in the dark at 27° for 18 h, inject a 10 μL aliquot. (Buffer was 100 mM pH 7.5 1-methylimidazole buffer. Synthesis of dansylethylenediamine is as follows. Add a solution of dansyl chloride in DMF to a 10% molar excess of ethylenediamine in DMF, evaporate to dryness, dissolve the residue in pH 10 carbonate buffer, purify by TLC, extract with MeOH.)

HPLC VARIABLES

Column: 250 × 4.6 Finepak ODP-50 octadecyl-bonded polyvinyl alcohol gel (Asahikasei, Tokyo)

Mobile phase: Gradient. A was MeCN:10 mM pH 10.3 phosphate buffer 12:88. B was MeCN:10 mM pH 10.3 phosphate buffer 22:78. C was MeCN:10 mM pH 10.3 phosphate buffer 40:60. A:B:C 100:0:0 for 10 min, to 0:100:0 over 18 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 10 min.

Column temperature: 40

Flow rate: 0.6

Injection volume: 10
Detector: F ex 270 em 546

CHROMATOGRAM
Retention time: 11
Limit of detection: 4.7-20.3 pmole

OTHER SUBSTANCES
Simultaneous: adenosine diphosphate, adenosine monophosphate, cytidine monophosphate, guanosine diphosphate, guanosine monophosphate, guanosine triphosphate, uridine monophosphate

KEY WORDS
derivatization

REFERENCE
Sonoki,S.; Sanda,A.; Hisamatsu,S. Simultaneous determination of mono-, di-, and trinucleotides by high-performance liquid chromatography using *N*-(dansyl)ethylenediamine as a fluorescent derivatizing reagent, *J.Liq.Chromatogr.*, **1994**, *17*, 1057–1064.

SAMPLE
Matrix: tissue
Sample preparation: Deproteinize heart tissue with ice-cold 600 mM perchloric acid, neutralize, centrifuge at 4° at 26890 g for 15 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 3 μm LC-18-T (Supelco)
Column: 150 × 4.6 3 μm LC-18-T (Supelco)
Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 1:99, pH 7.0. B was MeOH:100 mM KH_2PO_4 containing 2.8 mM tetrabutylammonium hydroxide 30:70, pH 5.5. A:B 100:0 for 12 min, to 60:40 over 2 min, to 56:44 over 11 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.2
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 45

OTHER SUBSTANCES
Extracted: adenosine, ascorbic acid

KEY WORDS
rat; heart

REFERENCE
Lazzarino,G.; Di Pierro,D.; Tavazzi,B.; Cerroni,L.; Giardina,B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography, *Anal.Biochem.*, **1991**, *197*, 191–196.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize 1 g frozen powdered tissue with 9 mL 2.5% perchloric acid. Neutralize a 200 μL aliquot with 2 M KOH containing 200 mM K_2HPO_4, centrifuge at 4° at 9500 g for 1 min, inject an aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm Hypersil
Column: 125 × 4.6 5 μm Hypersil 5ODS
Mobile phase: Gradient. A was 100 mM pH 6.0 potassium phosphate buffer containing 8 mM tetra-n-butylammonium hydrogen sulfate and 15 mM chloroacetaldehyde. B was MeCN:water 75:25. A:B from 97:3 to 65:35 over 6 min, return to initial conditions over 1 min.
Flow rate: 1.6
Detector: F ex 230 em 430 following post-column reaction. The column effluent flowed through a knitted 10 m × 0.5 mm ID PTFE coil at 100° to the detector.

CHROMATOGRAM

Retention time: 8.5
Limit of detection: 10 pmole

OTHER SUBSTANCES

Extracted: adenosine diphosphate, adenosine monophosphate

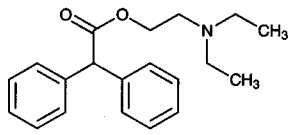
KEY WORDS

post-column reaction; mouse; liver

REFERENCE

Stratford,M.R.L.; Dennis,M.F. Determination of adenine nucleotides by fluorescence detection using high-performance liquid chromatography and post-column derivatization with chloroacetaldehyde, *J.Chromatogr.B*, **1994**, *662*, 15–20.

Adiphenine



Molecular formula: $C_{20}H_{25}NO_2$
Molecular weight: 311.42
CAS Registry No.: 64-95-9, 50-42-0 (HCl), 6113-04-8 (methyl bromide)
Merck Index: 160

SAMPLE
Matrix: solutions

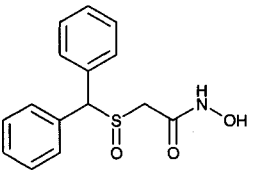
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 250 × 4 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 260

CHROMATOGRAM
Retention time: k' 2.3

OTHER SUBSTANCES
Simultaneous: diphenylacetic acid

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr. Rel.Technol.*, **1998**, *21*, 1347–1357.

Adrafinil



Molecular formula: $C_{15}H_{15}NO_3S$
Molecular weight: 289.36
CAS Registry No.: 63547-13-7
Merck Index: 168

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.833

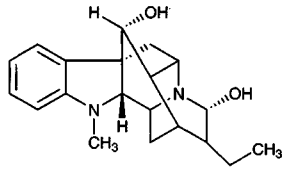
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ajmaline



Molecular formula: $C_{20}H_{26}N_2O_2$
Molecular weight: 326.44
CAS Registry No.: 4360-12-7
Merck Index: 194

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 246

CHROMATOGRAM

Retention time: 4.37

Limit of detection: <120 ng/mL

KEY WORDS

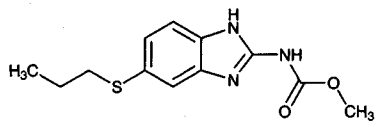
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Albendazole



Molecular formula: $C_{12}H_{15}N_3O_2S$
Molecular weight: 265.34
CAS Registry No.: 54965-21-8
Merck Index: 211
Lednicer No.: 2 353

SAMPLE

Matrix: abomasal fluid, blood, duodenal fluid, rumen fluid
Sample preparation: 4 mL Plasma, rumen fluid, abomasal fluid, or duodenal fluid + 4 mL pH 7.4 phosphate buffer + 20 mL ether, shake on a rotary mixer for 10 min, remove 16 mL of the ether layer, add 20 mL ether, shake on a rotary mixer for 10 min, remove 20 mL of the ether layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: oxfendazole, thiabendazole, cambendazole, mebendazole, oxibendazole, fenbendazole, parbendazole

KEY WORDS

plasma; sheep

REFERENCE

Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

SAMPLE

Matrix: blood
Sample preparation: 200-1000 μL Plasma + 150 ng proguanil hydrochloride (in water) + 200-1000 μL MeCN, vortex for 5 s, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 1 mL water, add 10 mL dichloromethane, vortex for 10 s, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 10-50 μL aliquot.

HPLC VARIABLES

Guard column: 10 μm CM Guard-Pak (Waters)
Column: 100 × 8 10 μm μBondapak phenyl
Mobile phase: MeOH:MeCN:1% triethylamine 10:20:70
Flow rate: 2.5
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.9
Internal standard: proguanil (5.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Hoaksey,P.E.; Awadzi,K.; Ward,S.A.; Coventry,P.A.; Orme,M.L'E.; Edwards,G. Rapid and sensitive method for the determination of albendazole and albendazole sulphoxide in biological fluids, *J.Chromatogr.*, **1991**, *566*, 244–249.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 200 μL 500 mM ammonium hydroxide (to adjust pH to 11) + 200 mg NaCl + 5 mL distilled diethyl ether, roll for 15 min, remove 4 mL supernatant, repeat extraction, remove 5 mL supernatant. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 60 μL MeOH, sonicate for 2 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 5 Nucleosil 5C18
Mobile phase: MeCN:1% acetic acid 43:57
Flow rate: 0.9
Injection volume: 20
Detector: UV 292

CHROMATOGRAM
Retention time: 1.6
Internal standard: albendazole

OTHER SUBSTANCES
Extracted: febantel, fenbendazole, oxfendazole

KEY WORDS
plasma; albendazole is IS; oxfendazole sulfone; sheep

REFERENCE
Landuyt,J.; Debackere,M.; Delbeke,F.; McKellar,Q. A high performance liquid chromatographic method for the determination of febantel and its major metabolites in lamb plasma, *Biomed.Chromatogr.*, **1993**, *7*, 78–81.

SAMPLE
Matrix: blood, CSF
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL 17 mM KH_2PO_4 adjusted to pH 5.5 with 800 mM NaOH. 2 mL Plasma or CSF + 100 μL 5 μg/mL mebendazole in MeOH + 2 mL 10 mM KH_2PO_4 adjusted to pH 7.4 with 800 mM NaOH, vortex for 30 s, add to the SPE cartridge, wash with 20 mL 10 mM KH_2PO_4 adjusted to pH 7.4 with 800 mM NaOH, wash with 1 mL MeOH:water 20:80, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS C18
Mobile phase: MeOH:50 mM pH 5.7 phosphate buffer 70:30
Flow rate: 0.8
Injection volume: 20
Detector: UV 295

CHROMATOGRAM
Retention time: 11.6
Internal standard: mebendazole (8.0)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Hurtado,M.; Medina,M.T.; Sotelo,J.; Jung,H. Sensitive high-performance liquid chromatographic assay for albendazole and its main metabolite albendazole sulphoxide in plasma and cerebrospinal fluid, *J.Chromatogr.*, **1989**, *494*, 403–407.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize lung tissue in 5 volumes 100 mM pH 6.0 Na_2HPO_4 and centrifuge at 1500 g for 15 min. 500 μL Serum or 1 mL lung tissue homogenate supernatant + 100 μL 3 μg/mL mebendazole in MeOH:DMSO 90:10 + 1 mL 100 mM potassium carbonate + 4 mL dichloromethane, mix on an Eberbach shaker for 10 min, centrifuge at 1500 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeOH:MeCN:70 mM monochloroacetic acid 27:18:55
Flow rate: 1.2
Injection volume: 10
Detector: UV 290

CHROMATOGRAM
Internal standard: mebendazole

KEY WORDS
serum; lung; mouse

REFERENCE
Bartlett,M.S.; Edlind,T.D.; Lee,C.H.; Dean,R.; Queener,S.F.; Shaw,M.M.; Smith,J.W. Albendazole inhibits Pneumocystis carinii proliferation in inoculated immunosuppressed mice, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1834–1837.

SAMPLE
Matrix: blood, urine
Sample preparation: 100-200 μL Whole blood, plasma, or urine + 1 mL 200 ng/mL mebendazole in ethyl acetate, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, inject.

HPLC VARIABLES
Column: 250 × 4.6 normal phase 5 μm Partisil
Mobile phase: Hexane:EtOH 89:11
Flow rate: 2
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 4.7
Internal standard: mebendazole (7.0)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; sheep; normal phase; pharmacokinetics

REFERENCE
Galtier,P.; Alvinerie,M.; Steimer,J.L.; Francheteau,P.; Plusquellec,Y.; Houin,G. Simultaneous pharmacokinetic modeling of a drug and two metabolites: application to albendazole in sheep, *J.Pharm.Sci.*, **1991**, *80*, 3–10.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM
Retention time: 17.777

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve sample in MeOH containing 10% formic acid, dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 19:81, pH 3.9 (Buffer was prepared by dissolving 6.6 g dibasic ammonium phosphate in 1 L water and adjusting to pH 3.9 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: fenbendazole, niclosamide, oxyclozanide

KEY WORDS
tablets; powder; liquid formulations

REFERENCE
van Tonder,E.C.; de Villiers,M.M.; Handford,J.S.; Malan,C.E.P.; Du Preez,J.L. Simple, robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations, *J.Chromatogr.A*, **1996**, *729*, 267–272.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Place 1.5 mL microsomal incubation in a boiling water bath for 2 min, add to a Sep-Pak C18 SPE cartridge, add mebendazole to the SPE cartridge, wash with water, elute with MeOH, inject an aliquot of the eluate.

HPLC VARIABLES
Column: Nucleosil C18
Mobile phase: Gradient. MeCN:0.5% acetic acid 35:65 for 5 min, 70:30 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1 for 5 min, 1.5 for 5 min, re-equilibrate at 1
Detector: UV 292

CHROMATOGRAM
Retention time: 8.2
Internal standard: mebendazole (7.3)
Limit of detection: 20 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; intestine; SPE

REFERENCE
Villaverde,C.; Alvarez,A.I.; Redondo,P.; Voces,J.; del Estal,J.L.; Prieto,J.G. Small intestinal sulphoxidation of albendazole, *Xenobiotica*, **1995**, *25*, 433–441.

SAMPLE
Matrix: milk

Sample preparation: 1 mL Milk + 100 μL 400 mM NaOH, extract with 8 mL ethyl acetate, vortex at high speed for 3 s, centrifuge at 4000 g for 2 min. Remove a 6 mL aliquot of the clear supernatant, add 2 mL water, vortex for 10 s, centrifuge at 1000 g for 30 s. Remove the organic layer and evaporate it to dryness under nitrogen at 40°. Reconstitute the residue in 500 μL mobile phase, filter (0.2 μm), inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:10 mM phosphoric acid 20:80 containing 5 mM tetrabutylammonium hydrogen sulfate
Column temperature: 50
Flow rate: 1
Injection volume: 100
Detector: UV 292

CHROMATOGRAM

Retention time: 12.5
Limit of detection: 5 ng/mL
Limit of quantitation: 1.6 ng

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: amoxicillin, ampicillin, chlortetracycline, cloxacillin, febantel, febendazole, febendazole hydroxide, febendazole sulfone, gentamicin, kanamycin, mebendazole, neomycin, oxacillin, oxfendazole, oxibendazole, oxytetracycline, penicillin G, penicillin V, streptomycin, tetracycline, thiabendazole, thiabendazole hydroxide, triclabendazole

KEY WORDS

milk; cow; pharmacokinetics

REFERENCE

Fletouris,D.J.; Boytsoglou,N.A.; Psomas,I.E.; Mantis,A.I. Trace analysis of albendazole and its sulphoxide and sulphone metabolites in milk by liquid chromatography, *J.Chromatogr.B*, **1996**, *687*, 427–435.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 45:55 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 305

OTHER SUBSTANCES

Also analyzed: epirizole, prochlorperazine

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: tissue

Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material, 0.5 g liver, and 10 μL 40 μg/mL mebendazole in DMF in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Retention time: 9.5
Internal standard: mebendazole (9)
Limit of detection: 100 ng/g

OTHER SUBSTANCES

Extracted: thiabendazole, oxfendazole, fenbendazole

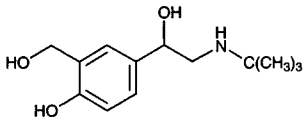
KEY WORDS

matrix solid-phase dispersion; liver

REFERENCE

Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

Albuterol



Molecular formula: $C_{13}H_{21}NO_3$
Molecular weight: 239.31
CAS Registry No.: 18559-94-9, 51022-70-9 (sulfate)
Merck Index: 217
Lednicer No.: 2 43

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL mobile phase , mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: F ex 222 em 300

CHROMATOGRAM

Retention time: 20.6
Internal standard: albuterol

OTHER SUBSTANCES

Extracted: atenolol
Noninterfering: chlorthalidone, xipamide

KEY WORDS

plasma; albuterol is IS

REFERENCE

Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE

Matrix: solutions
Sample preparation: Dilute 800 μL solution to 10 mL with water, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: RP-18
Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:buffer 4:96 for 6 min, to 9:91 (step gradient). (Buffer was 40 mM NaH_2PO_4 containing 5.74 mM triethylamine, adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 265

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Mälkki-Laine,L.; Hartikainen,E. Electrokinetic behaviour of salbutamol and its decomposition products and determination of salbutamol by micellar electrokinetic capillary chromatography, *J.Chromatogr.A*, **1996**, *724*, 297–306.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 CSP-4 (Prepare as follows. Add a solution of 1.07 g L-valyl-L-valyl-L-valine isopropylester (Bunseki Kagaku 1079, 28, 125) in 30 mL dry dioxane (Caution! Dioxane is a carcinogen!) dropwise to a mixture of 2.2 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 20 mL dry dioxane stirred at 0°, add 3 g anhydrous sodium carbonate at room temperature, stir, filter, evaporate to give a colorless solid. Dissolve 8.3 g of this solid in 30 mL dry dioxane, add 2 g N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, add 1.5 g anhydrous sodium carbonate, reflux with stirring for 40 h, filter, add 3 g dried 10 μm LiChrosorb Si 100, reflux with slow stirring for 10 h, cool, filter. Wash the solid with dioxane, MeOH, and diethyl ether, dry under reduced pressure (J.Chromatogr. 1984, 292, 427).)
Mobile phase: Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 60:37.5:3.75:0.25
Detector: UV

CHROMATOGRAM
Retention time: k' 5.84 (first enantiomer)

KEY WORDS
chiral; α = 1.06

REFERENCE
Oi,N.; Kitahara,H.; Matsushita,Y.; Kisu,N. Enantiomer separation by gas and high-performance liquid chromatography with tripeptide derivatives as chiral stationary phases, *J.Chromatogr.A*, **1996**, *722*, 229–232.

Aldesleukin

Molecular formula: $C_{690}H_{1115}N_{177}O_{203}S_6$
Molecular weight: 15600
CAS Registry No.: 110942-02-4

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 μL sample with 225 μL MeOH and 125 μL chloroform. Add 125 μL 100 mM HCl and 125 μL chloroform. Mix thoroughly and centrifuge at 7500 g for 5 min. Inject a 100 μL aliquot of the water/MeOH layer. (Caution! Chloroform is a carcinogen!)

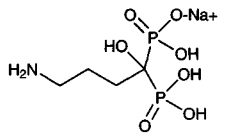
HPLC VARIABLES

Column: Phenomenex W–Porex C4
Mobile phase: MeCN:water containing 100 mM sodium perchlorate and 10 mM perchloric acid 52.5:47.5
Column temperature: 30
Injection volume: 100
Detector: UV 205

REFERENCE

Koppenhagen,F.J.; Visser,A.J.W.G.; Herron,J.N.; Storm,G.; Crommelin,D.J.A. Interaction of recombinant interleukin-2 with liposomal bilayers, *J.Pharm.Sci.*, **1998**, *87*, 707–714.

Alendronate sodium



Molecular formula: $C_4H_{12}NNaO_7P_2$
Molecular weight: 271.08
CAS Registry No.: 121268-17-5 (trihydrate), 66376-36-1 (free acid)
Merck Index: 228

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Condition an Analytichem 3 mL 500 mg diethylamine SPE cartridge with 3 mL water. 5 mL Urine + 50 µL 2.5 M calcium chloride, vortex, add 50 µL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 50 µL 1 M HCl, add 5 mL water, precipitate by adding 50 µL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 50 µL 1 M HCl, add 1 mL 10 mM EDTA, add 2 mL 100 mM pH 4.0 sodium acetate buffer, add to the SPE cartridge, wash with 3 mL water, elute with 3 mL buffer. Remove 250 µL of the eluate and add it to 250 µL 100 mM pH 9.1 sodium borate buffer, vortex, add 10 µL 50 mM KCN, add 10 µL 2 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, let stand for 15 min, inject a 100 µL aliquot. Plasma. Condition an Analytichem 3 mL 500 mg diethylamine SPE cartridge with 3 mL water. 1 mL Plasma + 1 mL 10% trichloroacetic acid, vortex, centrifuge at 5300 g for 10 min. Remove the supernatant and add it to 50 µL 100 mM sodium pyrophosphate in water, add 50 µL 2.5 M calcium chloride, vortex, add 50 µL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 50 µL 1 M HCl, add 5 mL water, precipitate by adding 50 µL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 50 µL 1 M HCl, add 1 mL 10 mM EDTA, add 2 mL 100 mM pH 4.0 sodium acetate buffer, add to the SPE cartridge, wash with 3 mL water, elute with 3 mL buffer. Remove 250 µL of the eluate and add it to 250 µL 100 mM pH 9.1 sodium borate buffer, vortex, add 10 µL 50 mM KCN, add 10 µL 2 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, let stand for 15 min, inject a 100 µL aliquot. (Buffer was 50 mM sodium citrate:50 mM pH 8.5 sodium phosphate buffer 1:1.)

HPLC VARIABLES

Guard column: 20 × 4.6 Hamilton PRP-1
Column: 150 × 4.6 5 µm 100 Å PLRP-S polymeric reversed-phase
Mobile phase: MeOH:buffer 40:60 (Buffer was 25 mM sodium citrate and 25 mM dihydrogenphosphate adjusted to pH 8.5 with 10 mM NaOH.)
Flow rate: 1
Injection volume: 100
Detector: F ex 420 em 490

CHROMATOGRAM

Retention time: 5
Limit of quantitation: 5 ng/mL (urine)

KEY WORDS

plasma; derivatization; SPE

REFERENCE

Kline,W.F.; Matuszewski,B.K.; Bayne,W.F. Determination of 4-amino-1-hydroxybutane-1,1-bisphosphonic acid in urine by automated pre-column derivatization with 2,3-naphthalene dicarboxaldehyde and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1990**, *534*, 139–149.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Condition an Analytichem 3 mL 200 mg diethylamine SPE cartridge with 3 mL water. 5 mL Urine + 50 μL 1.25 M calcium chloride, vortex, add 50 μL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 100 μL 1 M HCl, add 5 mL water, precipitate by adding 100 μL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 800 μL 200 mM acetic acid, add 400 μL 10 mM EDTA, add 400 μL 200 mM sodium acetate, add 3 mL water, add to the SPE cartridge, elute with 1 mL buffer. Remove 250 μL of the eluate and add it to 50 μL 1 M pH 10.7 carbonate buffer, vortex, add 10 μL 1 mg/mL N-acetyl-D-penicillamine in MeOH, add 10 μL 1 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, inject a 50 μL aliquot. Plasma. Condition an Analytichem 3 mL 200 mg diethylamine SPE cartridge with 3 mL water. 1 mL Plasma + 3 mL water + 250 μL 10% trichloroacetic acid, vortex, add three more 250 μL portions of 10% trichloroacetic acid with vortexing each time, centrifuge at 5300 g for 10 min. Remove the supernatant and add it to 200 μL 22 mM sodium pyrophosphate in water, add 50 μL 2.5 M calcium chloride, vortex, add 50 μL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 100 μL 1 M HCl, add 5 mL water, precipitate by adding 100 μL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 800 μL 200 mM acetic acid, add 400 μL 10 mM EDTA, add 400 μL 200 mM sodium acetate, add 3 mL water, add to the SPE cartridge, elute with 1 mL buffer. Remove 250 μL of the eluate and add it to 50 μL 1 M pH 10.7 carbonate buffer, vortex, add 10 μL 1 mg/mL N-acetyl-D-penicillamine in MeOH, add 10 μL 1 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, inject a 50 μL aliquot. (Buffer was 200 mM sodium citrate: 200 mM pH 8.5 Na_2HPO_4 buffer 1:1.)

HPLC VARIABLES

Guard column: 20 × 4.6 Hamilton PRP-1
Column: 150 × 4.6 5 μm 100 Å PLRP-S polymeric reversed-phase
Mobile phase: Gradient. MeCN:buffer at 15:85 for 10 min, to 32.5:67.5 over 5 min, return to initial conditions over 5 min, re-equilibrate for 10 min. (Buffer was 25 mM sodium citrate and 25 mM dihydrogenphosphate adjusted to pH 6.3 with 85% phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: F ex 436 em 440 (cut-off filter)

CHROMATOGRAM

Retention time: 7
Limit of detection: 0.2 ng/mL (urine)
Limit of quantitation: 5 ng/mL (plasma), 1 ng/mL (urine)

KEY WORDS

plasma; derivatization; SPE

REFERENCE

Kline,W.F.; Matuszewski,B.K. Improved determination of the bisphosphonate alendronate in human plasma and urine by automated precolumn derivatization and high-performance liquid chromatography with fluorescence and electrochemical detection, *J.Chromatogr.*, **1992**, *583*, 183–193.

SAMPLE

Matrix: formulations
Sample preparation: Dilute injections to a concentration of 25 μg/mL with 100 mM sodium citrate. Shake 1 tablet or the contents of 1 capsule with 50 mL 100 mM sodium citrate for 30 min, sonicate for 5 min, make up to 100 mL with 100 mM sodium citrate, dilute to 25 μg/mL, centrifuge at 2000 rpm for 10 min. Mix 5 mL supernatant or diluted injection with 5 mL 100 mM pH 9.0 sodium borate, add 4 mL 500 μg/mL 9-fluorenylmethyl chloroformate in MeCN, vortex for 30 s, let stand for 30 min, add 25 mL dichloromethane, shake for 30-60 s, let stand for 5 min, centrifuge at 1000 rpm for 5 min, inject a 50 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: 250 × 4.1 10 μm PRP-1 (Hamilton)
Mobile phase: MeCN:MeOH:buffer 20:5:75 (Prepare buffer by dissolving 14.7 g sodium citrate dihydrate and 8.7 g K_2HPO_4 in 900 mL water, adjust pH to 8.0 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 266

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
derivatization; injections; capsules; tablets

REFERENCE
De Marco,J.D.; Biffar,S.E.; Reed,D.G.; Brooks,M.A. The determination of 4-amino-1-hydroxybutane-1,1-diphosphonic acid monosodium salt trihydrate in pharmaceutical dosage forms by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1719–1727.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Simultaneous: clodronate, etidronate, neridronate, olpadronate, pamidronate

KEY WORDS
derivatization; complexation; injections; tablets

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1545–1550.

SAMPLE
Matrix: formulations
Sample preparation: Stir tablets containing 40 mg alendronate in 100 mL water for 30 min, filter (0.22 μm) an aliquot, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 6 μm IC-Pak HR polymethacrylate quaternary ammonium anion-exchange (Waters)
Mobile phase: 6 mM nitric acid
Column temperature: 25
Flow rate: 0.5
Injection volume: 50
Detector: RI

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 400 ng/mL

KEY WORDS
tablets

REFERENCE
Han,Y.-H.R.; Qin,X.-Z. Determination of alendronate sodium by ion chromatography with refractive index detection, *J.Chromatogr.A*, **1996**, *719*, 345–352.

SAMPLE
Matrix: urine
Sample preparation: Condition an Analytichem 3 mL 200 mg diethylamine SPE cartridge with 3 mL water. 5 mL Urine + 50 μL 2.5 M calcium chloride, vortex, add 50 μL or more (as needed) 1 M NaOH to form a slight white precipitate, centrifuge. Discard supernatant and dissolve pellet in 50 μL 1 M HCl, add 5 mL water, precipitate by adding 50 μL 1 M NaOH, centrifuge. Discard the supernatant and dissolve the pellet in 50 μL 1 M HCl, add 1 mL 10 mM EDTA, add 2 mL 100 mM pH 4.0 sodium acetate buffer, add to the SPE cartridge, wash with 3 mL water, elute with 3 mL buffer. Remove 250 μL of the eluate and add it to 50 μL 1 M pH 10.7 carbonate buffer, vortex, add 10 μL 50 mM KCN, add 10 μL 2 mg/mL 2,3-naphthalenedicarboxaldehyde in MeOH, let stand for 15 min, inject a 100 μL aliquot. (Buffer was 50 mM sodium citrate:50 mM pH 8.5 sodium phosphate buffer 1:1.)

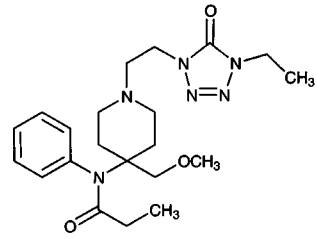
HPLC VARIABLES
Guard column: 20 × 4.6 Hamilton PRP-1
Column: 150 × 4.6 5 μm 100 Å PLRP-S polymeric reversed-phase
Mobile phase: MeOH:buffer 40:60 (Buffer was 25 mM sodium citrate and 25 mM dihydrogenphosphate adjusted to pH 8.5 with 10 mM NaOH.)
Flow rate: 1
Injection volume: 100
Detector: E, LC-4B (Bioanalytical Systems), LC-17A ED flow cell with glassy carbon electrode +0.65 V, RE-4 Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.4
Limit of quantitation: 2.5 ng/mL

KEY WORDS
derivatization; SPE

REFERENCE
Kline,W.F.; Matuszewski,B.K. Improved determination of the bisphosphonate alendronate in human plasma and urine by automated precolumn derivatization and high-performance liquid chromatography with fluorescence and electrochemical detection, *J.Chromatogr.*, **1992**, *583*, 183–193.

Alfentanil



Molecular formula: $C_{21}H_{32}N_6O_3$
Molecular weight: 416.52
CAS Registry No.: 71195-58-9, 69049-06-5 (HCl), 70879-28-6 (HCl monohydrate)
Merck Index: 236
Lednicer No.: 3 118

SAMPLE

Matrix: blood, urine
Sample preparation: 50 µL Plasma or urine + 50 µL 4 M NaOH + 100 µL MeCN + 500 µL n-hexane, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 µm Nova pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 5.78
Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Extracted: fentanyl, sufentanil

KEY WORDS

plasma

REFERENCE

Bansal,R.; Aranda,J.V. Simultaneous microassay of alfentanil, fentanil, and sufentanil by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 339–348.

SAMPLE

Matrix: cell cultures
Sample preparation: Freeze a 400 µL aliquot of the hepatocyte culture in hexane/dry ice, add an equal volume of DMSO, thaw, vortex, sonicate for 5 s, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 5 µm Hypersil C18
Mobile phase: Gradient. A was 100 mM pH 7.0 ammonium acetate. B was MeCN:MeOH:THF:1 M pH 7.0 ammonium acetate 30:20:40:10. A:B from 100:0 to 40:60 over 40 min.
Flow rate: 1
Injection volume: 200
Detector: UV 230

CHROMATOGRAM

Retention time: 40

OTHER SUBSTANCES

Extracted: metabolites

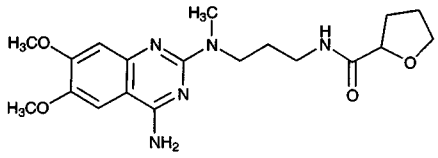
KEY WORDS

hepatocyte cultures; rat; dog

REFERENCE

Lavrijsen,K.; Van Houdt,J.; Meuldermans,W.; Knaeps,F.; Hendrickx,J.; Lauwers,W.; Hurkmans,R.; Heykants,J. Metabolism of alfentanil by isolated hepatocytes of rat and dog, *Xenobiotica*, **1988**, *18*, 183–197.

Alfuzosin



Molecular formula: $C_{19}H_{27}N_5O_4$
Molecular weight: 389.45
CAS Registry No.: 81403-80-7, 81403-68-1 (HCl)
Merck Index: 237
Lednicer No.: 4 149

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 2 mg/mL IS in MeOH, vortex, add 1 mL 100 mM NaOH, add 7 mL dichloromethane:diethyl ether (nonstabilized) 3:4, stir for 25 min. Centrifuge at 1000 g at 4° for 5 min, remove the organic layer, evaporate it to dryness under a stream of nitrogen at 30°. Dissolve the residue in 80 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm Chiral-AGP (ChromTech)
Mobile phase: MeCN:buffer 6:94 (Buffer was 50 mM KH_2PO_4 adjusted to pH 7.4 with 1 M NaOH.)
Flow rate: 0.9
Injection volume: 20
Detector: F ex 265 em 400

CHROMATOGRAM

Retention time: 4 (R), 6 (S)
Limit of detection: 1 ng/mL

KEY WORDS

chiral; plasma

REFERENCE

Krstulovic,A.M.; Vende,J.L. Improved performance of the second generation α 1-AGP columns: applications to the routine assay of plasma levels of alfuzosin hydrochloride, *Chirality*, **1989**, *1*, 243–245.

SAMPLE

Matrix: blood
Sample preparation: Dilute 100 μL urine to 1 mL with water. 1 mL PLasma, whole blood, or diluted urine + 10 μL 5 μg/mL IS in water + 1 mL 100 mM NaOH + 7 mL diethyl ether, shake for 30 min, centrifuge at 4° at 1000 g for 5 min. Remove 6.5 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 870 μL MeCN:20 mM pH 2.5 KH_2PO_4 10:90, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:20 mM pH 2.5 KH_2PO_4 60:40
Flow rate: 1
Injection volume: 500
Detector: F ex 334 em 378

CHROMATOGRAM

Retention time: 4.4
Internal standard: N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)amino]propyl]-N-methyltetrahydro-2H-pyran-2-carboxamide hydrochloride (5.6)
Limit of detection: 0.5 ng/mL

KEY WORDS
plasma; whole blood

REFERENCE
Guinebault,P.; Broquaire,M.; Colafranceschi,C.; Thénot,J.P. High-performance liquid chromatographic determination of alfuzosin in biological fluids with fluorimetric detection and large-volume injection, *J.Chromatogr.*, **1986**, *353*, 361–369.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 2 μg/mL IS in MeOH + 1 mL 100 mM NaOH + 7 mL dichloromethane:diethyl ether 3:4, shake for 25 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 80 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm chiral-AGP (ChromTech)
Mobile phase: MeCN:buffer 6:94, pH 7.4 (Buffer was 0.667 g KH_2PO_4, 4.587 g $K_2HPO_4.3H_2O$, and 8.060 g tetrabutylammonium bromide in 1 L water.)
Flow rate: 0.9
Injection volume: 20
Detector: F ex 265 em 400

CHROMATOGRAM
Retention time: 5.32 (R), 6.58 (S)
Internal standard: N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]-4,5-dihydro-2-furancarboxamide (9.75)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: furosemide, zolpidem
Noninterfering: betaxolol, captopril, diazepam, enalapril, metoprolol, piroxicam, propranolol, warfarin

KEY WORDS
chiral; plasma; pharmacokinetics

REFERENCE
Rouchouse,A.; Manoha,M.; Durand,A.; Thenot,J.P. Direct high-performance liquid chromatographic determination of the enantiomers of alfuzosin in plasma on a second-generation α 1-acid glycoprotein chiral stationary phase, *J.Chromatogr.*, **1990**, *506*, 601–610.

SAMPLE
Matrix: blood
Sample preparation: Inject 50 μL plasma on to column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A on to column B with mobile phase B, after 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 50 × 4.6 10 μm LiChrosorb C18; B 20 × 4.6 30 μm Pellicular-CN (Merck) + 250 × 4.6 5 μm Spherisorb S5W cyanopropyl
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:50 mM pH 2.5 phosphate buffer 38:2:60
Flow rate: 1
Injection volume: 50
Detector: F ex 265 em 400

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 1 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Carlucci,G.; Di Giuseppe,E.; Mazzeo,P. Determination of alfuzosin in human plasma by high-performance liquid chromatography with column-switching, *J.Liq.Chromatogr.*, **1994**, *17*, 3989–3997.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

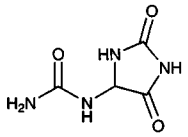
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 244

CHROMATOGRAM
Retention time: 10.37

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Allantoin



Molecular formula: $C_4H_6N_4O_3$
Molecular weight: 158.12
CAS Registry No.: 97-59-6
Merck Index: 255

SAMPLE

Matrix: blood
Sample preparation: Cool 1 mL plasma in an ice bath, add 100 μL 1.1 mM allopurinol in water, add 100 μL 4 M perchloric acid, vortex for 1 min, cool in an ice bath for 10 min, centrifuge at 10000 g for 5 min. Remove the supernatant and adjust the pH to 6-7 with ice-cold 4 M KOH using bromothymol blue as an indicator, let stand for 10 min, centrifuge at 2000 g for 10 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-5
Mobile phase: Gradient. A was 10 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid. B was MeCN:12.5 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid 20:80. A:B at 100:0 for 5 min, to 98:2 over 2 min, to 0:100 over 23 min, re-equilibrate at 100:0 for 20 min.
Flow rate: 0.8
Detector: UV 205

CHROMATOGRAM

Retention time: 3
Internal standard: allopurinol (16)
Limit of detection: 0.06 nmole

OTHER SUBSTANCES

Extracted: metabolites, uric acid, xanthine, hypoxanthine

KEY WORDS

plasma; sheep

REFERENCE

Balcells,J.; Guada,J.A.; Peiró,J.M.; Parker,D.S. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 153–157.

SAMPLE

Matrix: blood
Sample preparation: 300 μL Plasma + 600 μL MeCN, mix vigorously, centrifuge at 2500 g for 10 min, remove the supernatant, add 600 μL MeCN:water 2:1 to the residue, mix vigorously, centrifuge. Combine the supernatants and add them to the SPE cartridge, recover the eluate, elute with 2 mL MeCN:1 mM phosphoric acid 50:50. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL 100 mM NaOH, heat on a boiling water bath for 20 min, cool, add 600 μL 1.5 mM 2,4-dinitrophenylhydrazine in 2.5 M HCl, heat at 50° for 1 h, centrifuge at 10000 g for 30 min, inject a 50 μL aliquot of the supernatant. (Prepare the SPE cartridges by making a slurry of AG1-X8 (chloride form) strong ion-exchange resin (Bio-Rad) in water, let stand for 30 min, add 0.5 mL of the slurry to a 65 × 5 polypropylene column fitted with a 20 μm polyethylene frit, add another frit to the top, wash the column with water until no chloride ions are eluted, equilibrate with 3 mL MeCN:1 mM phosphoric acid 50:50. Test for the presence of chloride ions by adding 1 mL eluate to 300 μL 300 mM silver nitrate solution, a white precipitate indicates the presence of chloride ions.

Regenerate columns by washing with 5 mL 1 M HCl, wash with 20 mL water until no chloride ions are detected.)

HPLC VARIABLES

Guard column: Reversed-phase guard column (Whatman)
Column: 100 × 4.6 Partisphere C18 (Whatman)
Mobile phase: Gradient. A was MeCN:100 mM pH 6.0 KH_2PO_4 5:95. B was MeCN:100 mM pH 6.0 KH_2PO_4 50:50. A:B 100:0 for 1 min, to 84:16 over 20 min, to 0:100 over 9 min, re-equilibrate at the initial conditions for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 360

CHROMATOGRAM

Retention time: 16.6
Limit of quantitation: 4 μM

KEY WORDS

plasma; SPE; derivatization

REFERENCE

Lagendijk,J.; Ubbink,J.B.; Vermaak,W.J.H. The determination of allantoin, a possible indicator of oxidant status, in human plasma, *J.Chromatogr.Sci.*, **1995**, *33*, 186–193.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 3 mL 10% trichloroacetic acid (cooled to 2-4°) to 3 mL plasma (cooled to 0-4°) or urine acidified to pH ≤3 with 1 M sulfuric acid and diluted 20 or 40 times with water, centrifuge at 4000 g at 4° for 15 min. Add a 500 μL aliquot of the supernatant to 50 μL 0.04% thymol blue pH indicator. If the reaction mixture color is orange (pH about 1.3) add 50 μL 600 mM NaOH, if the color fails to change to blue add more 600 mM NaOH. When the color changes to blue (pH 9.3-11) heat the reaction mixture at 85° for 60 min. Add 200 μL derivatizing solution (the color becomes orange-yellow), continue the heating for 20 min, filter (0.2 μm), inject an aliquot of the filtrate. (Prepare the derivatizing solution by dissolving 100 mg 2,4-dinitrophenylhydrazine in 100 mL 2 M HCl and by filtering through a membrane filter.)

HPLC VARIABLES

Guard column: 10 × 6 30-40 μm C18 pellicular material (Waters)
Column: two 250 × 4.6 4 μm Nova-Pak C18 columns in series
Mobile phase: Gradient. A was 2.5 mM ammonium dihydrogen phosphate adjusted to pH 3.5 with 10% H_3PO_4. B was MeCN:2.5 mM pH 3.5 ammonium dihydrogen phosphate 20:80. A:B 100:0 for 7.7 min, to 0.8:99.2 in 0.6 min, from 0.8:99.2 to 0.4:99.6 in 8.7 min, from 0.4:99.6 to 100:0 in 31.5 min, maintain at 100:0 for 11.5 min.
Flow rate: 0.9 for 17.1 min, 1 for 3.9 min, 1.2 for 39 min
Injection volume: 10
Detector: UV 205 for 9 min, UV 254 for 16 min, then UV 360

CHROMATOGRAM

Retention time: 37.6
Limit of detection: 4 pmol

OTHER SUBSTANCES

Extracted: uric acid, hypoxanthine, xanthine

KEY WORDS

plasma; sheep; urine

REFERENCE

Czauderna,M.; Kowalczyk,J. Simultaneous measurement of allantoin, uric acid, xanthine and hypoxanthine in blood by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *704*, 89–98.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Adjust pH to < 3 with 1 M sulfuric acid, dilute so that the concentration of allantoin was 0.03-0.32 mM. 500 μL + 50 μL indicator + 100 μL 600 mM NaOH, if the color did not change to blue (pH > 9.2) add 600 mM NaOH in 50 μL increments, heat at 85° for 1 h, add 200 μL reagent, heat at 85° for 20 min, cool, centrifuge at 35000 g for 15 min, inject a 10 μL aliquot of the supernatant. Plasma. 2 mL Plasma + 2 mL 10% trichloroacetic acid, centrifuge at 35000 g for 20 min. 500 μL supernatant + 50 μL indicator + 100 μL 600 mM NaOH, if the color did not change to blue (pH > 9.2) add 600 mM NaOH in 50 μL increments, heat at 85° for 1 h, add 200 μL reagent, heat at 85° for 20 min, cool, centrifuge at 35000 g for 15 min, inject a 10 μL aliquot of the supernatant. (Indicator was 600 mM NaOH and 0.04% thymol blue in water, filter (Whatman No. 1 paper). Reagent was 1 g/L 2,4-dinitrophenylhydrazine in 2 M HCl, filter (Whatman No. 1 paper).)

HPLC VARIABLES

Guard column: 25 × 2 30-40 μm pellicular reversed-phase C18

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was MeCN:10 mM acetic acid, pH adjusted to 6.1 with ammonia 15:85. B was MeCN. A:B from 100:0 to 82:18 over 3 min (Waters concave no. 7) to 18:82 over 2 min (Waters concave no. 7), stay at 18:82 for 3 min, to 100:0 over 1 min (linear), re-equilibrate at 100:0 for 15 min.

Flow rate: 1

Injection volume: 10

Detector: UV 360

CHROMATOGRAM

Retention time: 3, 7.3 (syn- and anti-derivatization products)

Limit of quantitation: 5000 nM

KEY WORDS

plasma; derivatization

REFERENCE

Chen,X.B.; Kyle,D.J.; Orskov,E.R. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1993**, *617*, 241–247.

SAMPLE

Matrix: urine

Sample preparation: Adjust pH to < 3 with sulfuric acid, dilute 1:20 with 100 mM $(NH_4)H_2PO_4$, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-5

Mobile phase: Gradient. A was 10 mM $(NH_4)H_2PO_4$, adjusted to pH 4.0 with phosphoric acid. B was MeCN:12.5 mM $(NH_4)H_2PO_4$, adjusted to pH 4.0 with phosphoric acid 20:80. A:B from 100:0 to 0:100 over 30 min, stay at 0:100 for 10 min, re-equilibrate at 100:0 for 20 min.

Flow rate: 0.8

Injection volume: 20

Detector: UV 205

CHROMATOGRAM

Retention time: 3

Limit of detection: 0.06 nmole

OTHER SUBSTANCES
Extracted: metabolites, uric acid, hypoxanthine

KEY WORDS
sheep

REFERENCE
Balcells,J.; Guada,J.A.; Peiró,J.M.; Parker,D.S. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 153–157.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.45 μm), dilute 10-fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: 10 mM pH 4.0 potassium phosphate buffer
Column temperature: 25
Flow rate: 0.5
Injection volume: 20
Detector: UV 218

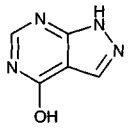
CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 0.2 mg/mL

OTHER SUBSTANCES
Extracted: creatinine

KEY WORDS
sheep

REFERENCE
Resines,J.A.; Díez,M.T.; Arín,M.J. Statistical evaluation of agreement between HPLC and colorimetric methods for analysis of allantoin in ruminants' urine, *J.Liq.Chromatogr.*, **1993**, *16*, 2853–2859.

Allopurinol



Molecular formula: $C_5H_4N_4O$
Molecular weight: 136.11
CAS Registry No.: 315-30-0
Merck Index: 287
Lednicer No.: 1 152, 269

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 75 μL 10 μg/mL acetaminophen in water, mix, inject a 20 μL aliquot directly.

HPLC VARIABLES

Guard column: 23 × 3.9 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 50 mM pH 6.0 phosphate buffer
Flow rate: 2.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 7.2
Internal standard: acetaminophen (18)
Limit of detection: 150 ng/mL

OTHER SUBSTANCES

Extracted: uric acid, oxipurinol

KEY WORDS

plasma; renew guard column after 50-70 injections

REFERENCE

Nissen,P. Simultaneous determination of allopurinol, oxipurinol and uric acid in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 382–386.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 102.7 μg/mL floxuridine in water, vortex 10 s, add 200 μL water containing 30% w/v trichloroacetic acid and 30% w/v perchloric acid, vortex 10 s, place in an ice bath for 2 min, centrifuge at 3000 g for 20 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 5μm Hypersil-ODS
Mobile phase: 20 mM Na_2HPO_4 adjusted to pH 2.0 with orthophosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: floxuridine (7)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: oxipurinol

KEY WORDS
plasma

REFERENCE
Hung,C.T.; Zoest,A.R.; Perrier,D.G. Analysis of allopurinol and oxipurinol in plasma by reversed phase HPLC, *J.Liq.Chromatogr.*, **1986**, *9*, 2471–2483.

SAMPLE
Matrix: blood
Sample preparation: Cool 1 mL plasma in an ice bath, add 100 μL 4 M perchloric acid, vortex for 1 min, cool in an ice bath for 10 min, centrifuge at 10000 g for 5 min. Remove the supernatant and adjust the pH to 6-7 with ice-cold 4 M KOH using bromothymol blue as an indicator, let stand for 10 min, centrifuge at 2000 g for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-5
Mobile phase: Gradient. A was 10 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid. B was MeCN:12.5 mM $(NH_4)H_2PO_4$, adjusted to pH 6.0 with phosphoric acid 20:80. A:B at 100:0 for 5 min, to 98:2 over 2 min, to 0:100 over 23 min, re-equilibrate at 100:0 for 20 min.
Flow rate: 0.8
Detector: UV 205

CHROMATOGRAM
Retention time: 16
Internal standard: allopurinol

OTHER SUBSTANCES
Extracted: allantoin, uric acid, xanthine, hypoxanthine

KEY WORDS
plasma; sheep; allopurinol is IS

REFERENCE
Balcells,J.; Guada,J.A.; Peiró,J.M.; Parker,D.S. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 153–157.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute plasma 1:2 (plasma) or 1:20 (urine) with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.1 5 μm SAS-Hypersil
Mobile phase: pH 7.0 Buffer containing 190 mL 100 mM citric acid, 810 mL 200 mM disodium phosphate, and 2 L water.
Flow rate: 2
Injection volume: 20
Detector: UV 252

CHROMATOGRAM
Retention time: 10
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: oxipurinol

Noninterfering: aminophylline, aspirin, 8-azaguanine, azathioprine, benzbromarone, caffeine, cotrimoxazole, cytarabine, diazepam, dihydralazine, dipyridamole, fluorouracil, guanine, hypoxanthine, 6-mercaptopurine, methotrexate, procarbazine, propranolol, spironolactone, sulfinpyrazone, 6-thioguanine, uric acid, xanthine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Breithaupt,H.; Goebel,G. Determination of allopurinol and oxipurinol in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *226*, 237–242.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. 0.5 mL Urine + 5 mL 20 mM pH 8.0 sodium phosphate buffer, mix, inject a 10-50 μL aliquot. Plasma. 0.5 mL Plasma + 0.4 mL water + 0.1 mL 20% perchloric acid, mix, centrifuge at 1300 g at 4° for 10 min. Mix 0.5 mL of the supernatant with 0.5 mL 200 mM disodium phosphate, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 300 × 3.9 8-10 μm μBondapak C18
Mobile phase: 4 mM pH 6.0 sodium phosphate buffer
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM

Retention time: 18.8
Limit of detection: 100-300 ng/mL

OTHER SUBSTANCES

Extracted: orotidine, orotic acid, uric acid, creatinine, hypoxanthine, xanthine, oxipurinol

KEY WORDS

plasma

REFERENCE

Miyazaki,H.; Matsunaga,Y.; Yoshida,K.; Arakawa,S.; Hashimoto,M. Simultaneous determination of plasma and urinary uric acid, xanthine, hypoxanthine, allopurinol, oxipurinol, orotic acid, orotidine and creatinine by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *274*, 75–85.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μL Plasma + 12.5 μM 9-methylxanthine + 150 μL 12% trichloroacetic acid, centrifuge, inject an aliquot. Urine. Inject directly.

HPLC VARIABLES

Guard column: 50 × 4.6 3 μm Hypersil ODS
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM KH_2PO_4 adjusted to pH 3.65 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: 9-methylxanthine
Limit of detection: 150 ng/mL

OTHER SUBSTANCES
Simultaneous: oxipurinol, xanthine, hypoxanthine

KEY WORDS
plasma

REFERENCE
Boulieu,R.; Bory,C.; Baltassat,P.; Gonnet,C. Simultaneous determination of allopurinol, oxipurinol, hypoxanthine and xanthine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *307*, 469–471.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount containing ca. 50 mg allopurinol, add 10 mL 100 mM NaOH, shake 10 min, dilute to 50 mL, filter (0.45 μm) discarding first 10 mL of filtrate. 4 mL Filtrate + 2 mL IS solution, make up to 200 mL with mobile phase, mix, inject a 10-20 μL aliquot. (Carry out sample preparation and injection without delay. IS solution was 50 mg hypoxanthine, add 10 mL 100 mM NaOH, shake 10 min, dilute to 50 mL. Prepare fresh daily.)

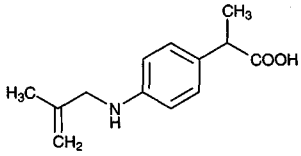
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak
Mobile phase: 50 mM Ammonium dihydrogen phosphate (For overnight storage flush system with water for at least 20 min and with MeOH for 20 min.)
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: hypoxanthine (7)

KEY WORDS
tablets

REFERENCE
Shostak,D. Liquid chromatographic determination of allopurinol in tablets: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1121–1122.

Alminoprofen



Molecular formula: $C_{13}H_{17}NO_2$
Molecular weight: 219.28
CAS Registry No.: 39718-89-3
Merck Index: 308

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 200 µg/mL glafenic acid in mobile phase + 100 µL 17 mM acetic acid + 7 mL diethyl ether, vortex for 10 s, centrifuge at 2000 g at 3° for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 µm µBondapak C18
Mobile phase: MeOH:water 50:50 with 1% glacial acetic acid
Flow rate: 0.85
Injection volume: 50
Detector: UV 235

CHROMATOGRAM

Retention time: 8
Internal standard: glafenic acid (5.6)
Limit of detection: 1000 ng/mL

KEY WORDS

plasma

REFERENCE

Paillet,M.; Merdjan,H.; Brouard,A.; Doucet,D.; Barreteau,H.; Fredj,G. Rapid determination of alminoprofen in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 455–459.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 253

CHROMATOGRAM

Retention time: 4.69
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, synovial fluid

Sample preparation: 1 mL Plasma + 100 μL saturated NaCl solution + 100 μL 17 mM acetic acid + ketoprofen + 7 mL diethyl ether, shake centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air with mild heating, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:water:acetic acid:DMSO 50:46:0.8:3
Flow rate: 1
Injection volume: 20
Detector: UV 251

CHROMATOGRAM
Retention time: 8.1
Internal standard: ketoprofen (11.7)
Limit of quantitation: 2 μg/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Tod,M.; Pobel,C.; Le Gros,V.; Louchahi,K.; Petitjean,O.; Brion,N.; Garcia-Macé,J.L. A population pharmacokinetic study of alminoprofen penetration into synovial fluid, *Biopharm.Drug Dispos.*, **1995**, *16*, 627–634.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

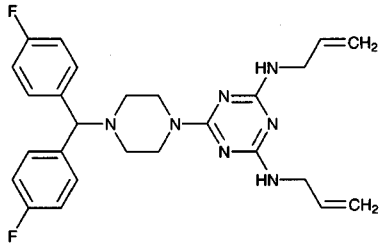
CHROMATOGRAM
Retention time: 18.695

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Almitrine

Molecular formula: $C_{26}H_{29}F_2N_7$
Molecular weight: 477.56
CAS Registry No.: 27469-53-0, 29608-49-9 (dimethanosulfonate)
Merck Index: 309



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 25.905

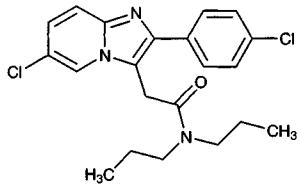
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Alpidem



Molecular formula: $C_{21}H_{23}Cl_2N_3O$
Molecular weight: 404.34
CAS Registry No.: 82626-01-5
Merck Index: 318

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently on a horizontal agitator for 10 min, centrifuge at 2800 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Buffer was saturated ammonium chloride, diluted 25% with water, adjusted to pH 9.5 with 25% diluted ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 10 mM KH_2PO_4 adjusted to pH 2.6 with orthophosphoric acid. At the end of the day wash column with water at 0.8 mL/min for 1 h and MeOH at 0.8 mL/min for 1 h.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 200
Detector: UV 247

CHROMATOGRAM

Retention time: 10.97
Limit of detection: 19.2 ng/mL

OTHER SUBSTANCES

Extracted: zopiclone, zolpidem, suriclone
Simultaneous: p-nitrophenol, ketotifen, tiaprofenic acid, vincristine, sultopride, pyrimethamine, nimodipine

KEY WORDS

plasma

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. High-performance liquid chromatographic assay with diode-array detection for toxicological screening of zopiclone, zolpidem, suriclone and alpidem in human plasma, *J.Chromatogr.*, **1993**, *616*, 95–103.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 2.5 μg/mL IS in MeOH, vortex, centrifuge at 11000 g for 4 min, inject a 200 μL aliquot onto column A with mobile phase A, elute with mobile phase A for 2 min, elute the contents of column A onto column B with mobile phase B for 1.5 min, remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B. Clean column A by back-flushing with MeCN: water 50:50, MeCN, MeOH:water 50:50, and water (all at 2 mL/min).

HPLC VARIABLES

Column: A 75 × 2.1 30-40 μm Perisorb C18; B 20 × 4.6 40 μm Pelliguard LC8 + 150 × 4.6 5 μm Hypersil BDS C8

Mobile phase: A water; B MeCN:MeOH:25 mM KH_2PO_4 40:15:45
Flow rate: 1.5
Injection volume: 200
Detector: F ex 255 em 423

CHROMATOGRAM
Retention time: 14.4
Internal standard: 6-chloro-2-(3,4-dimethoxyphenyl)-N,N-dipropylimidazo[1,2-a]pyridine-3-acetamide (SL 80.0633) (8)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: trazodone, zolpidem
Noninterfering: diazepam, nordiazepam, nitrazepam, lorazepam, cimetidine, amitriptyline, clomipramine, ranitidine

KEY WORDS
plasma; column-switching

REFERENCE
Flaminio,L.; Ripamonti,M.; Ascalone,V. Determination of alpidem, and imidazopyridine anxiolytic, and its metabolites by column-switching high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1994**, *668*, 403–411.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM
Retention time: 10.97
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine;

pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize brain tissue with 5 volumes water. 200-500 µL Plasma or brain homogenate + 1 mL 1 M pH 10 carbonate buffer + 20 µL 10 µg/mL IS in MeCN + 7 mL diethyl ether, agitate on a Bioblock REAX 3 agitator for 20 min, centrifuge at 1000 g at 4° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 120 µL MeCN:water 40:60, inject a 90 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb ODS
Mobile phase: MeCN:MeOH:10 mM pH 2.5 KH_2PO_4 50:10:40
Flow rate: 1
Injection volume: 90
Detector: F ex 257 em 398

CHROMATOGRAM

Internal standard: 6-chloro-2-(3,4-dimethoxyphenyl)-N,N-dipropyl-imidazo[1,2-a]pyridine-3-acetamide (SL 80.0633)
Limit of detection: 1 ng/mL

KEY WORDS

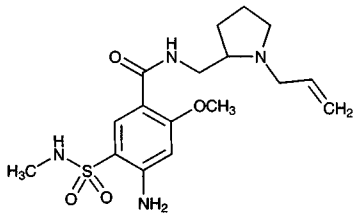
plasma; rat; brain

REFERENCE

Garrigou-Gadenne,D.; Durand,A.; Thenot,J.P.; Morselli,P.L. The disposition and pharmacokinetics of alpidem, a new anxiolytic, in the rat, *Drug Metab.Dispos.*, **1991**, *19*, 574–579.

Alpiropride

Molecular formula: $C_{17}H_{26}N_4O_4S$
Molecular weight: 382.48
CAS Registry No.: 81982-32-3
Merck Index: 319



SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a 3 mL C8 Analytichem SPE cartridge with 1 volume (2.7 mL) MeOH and 1 volume buffer A, do not allow to dry. Mix 1 mL plasma + 100 µL 10 µg/mL amisulpride in 10 mM HCl + 1 mL buffer A, add to SPE cartridge, rinse the sample container with 1 mL buffer A and add the rinse to the SPE cartridge, wash with 1 volume water, wash with 2 mL buffer B, dry the column for 1 min, wash with 200 µL acetone, dry for 30 s, elute with 1 mL buffer C, add 50 µL buffer D, evaporate to dryness under a stream of air, reconstitute in 200 µL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker 3 mL ion exchange quaternary aminesilicane-bonded silica gel SPE cartridge on top of a 3 mL Baker carboxylic acid-bonded silica gel SPE cartridge, condition with 1 volume (2.7 mL) buffer D, 1 volume of water, 1 volume of MeOH, and 1 volume of water. Mix 1 mL urine + 100 µL 10 µg/mL amisulpride in 10 mM HCl + 1 mL water, add to SPE cartridges, rinse the sample container with 2 mL water and add the rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash the bottom column with 1 volume of water and 2 volumes of MeOH, dry the column for 1 min, elute with 1 mL buffer D, evaporate the eluate to dryness under a stream of air at 45°, reconstitute in 200 µL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine + 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH (100 mM).)

HPLC VARIABLES

Guard column: 10 cm long Chrompack reverse-phase pellicular material
Column: 250 × 4.6 10 µm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 160:80:760 (Buffer was 10 mL triethylamine + 760 mL water adjusted to pH 6.8 with acetic acid (about 4.2 mL).)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM

Retention time: 6.0
Internal standard: amisulpride (4.9)

OTHER SUBSTANCES

Simultaneous: metoclopramide, alizapride, aspirin, theophylline, acetaminophen, caffeine, isosorbide-5-mononitrate, acenocoumarol, carbamazepine, nitrazepam, clonazepam, codeine, nitrofurantoin
Noninterfering: indomethacin, orphenadrine, furosemide, cisplatin, amitriptyline, isosorbide dinitrate, propranolol

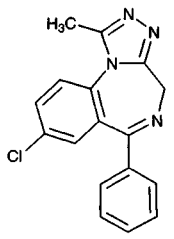
KEY WORDS

plasma; SPE; alpiropride is IS

REFERENCE

de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

Alprazolam



Molecular formula: $C_{17}H_{13}ClN_4$
Molecular weight: 308.77
CAS Registry No.: 28981-97-7
Merck Index: 320
Lednicer No.: 3 197

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 μL 512 nM IS in MeOH:water 5:95, add to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH. Elute with 200 μL and 100 μL MeOH, evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 4 μm Novapak C18
Mobile phase: MeCN:MeOH:10 mM pH 3.7 K_2HPO_4 30:2:100
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 8.5
Internal standard: flunitrazepam (9.8)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: clonazepam, nitrazepam
Simultaneous: amobarbital, carbamazepine, citalopram, clobazam, clozapine, diazepam, doxepin, ethosuximide, norclobazam, oxazepam, oxcarbamazepine, pentobarbital, phenobarbital, phenytoin, primidone, valproic acid, zopiclone
Interfering: medazepam, midazolam, nordiazepam, temazepam

KEY WORDS

SPE; plasma; serum

REFERENCE

Åkerman,K.K.; Jolkkonen,J.; Parviainen,M.; Penttilä,I. Analysis of low-dose benzodiazepines by HPLC with automated solid-phase extraction, *Clin.Chem.*, **1996**, *42*, 1412–1416.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeCN and 5 mL water. Mix 1 mL plasma with 10 μL 1 μg/mL IS in MeOH, dilute with 5 mL 1 M NaCl, mix briefly. Add to the SPE cartridge, wash with 10 mL water, elute with 5 mL MeCN:water 20:80, evaporate the eluate to dryness under reduced pressure at 60°, dissolve the residue in 50 μL MeOH and 100 μL mobile phase, inject an aliquot.; SPE

HPLC VARIABLES

Column: 150 × 4.6 5 μm Develosil C8-5 (Nomura Chemical, Seto, Japan)
Mobile phase: MeCN:0.5% pH 4.5 KH_2PO_4 30:70
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 17.5
Internal standard: estazolam (14.3)
Limit of detection: 500 pg/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Yasui,N.; Otani,K.; Kaneko,S.; Ohkubo,T.; Osanai,T.; Sugawara,K.; Chiba,K.; Ishizaki,T. A kinetic and dynamic study of oral alprazolam with and without erythromycin in humans: In vivo evidence for the involvement of CYP3A4 in alprazolam metabolism, *Clin.Pharmacol.Ther.*, **1996**, *59*, 514–519.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

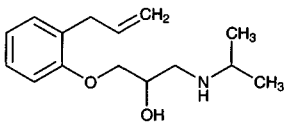
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 16.972

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Alprenolol



Molecular formula: $C_{15}H_{23}NO_2$
Molecular weight: 249.35
CAS Registry No.: 13655-52-2, 13707-88-5 (HCl)
Merck Index: 321
Lednicer No.: 1 177

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 270

CHROMATOGRAM

Retention time: 6.18
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH, dilute with mobile phase.

HPLC VARIABLES

Column: 150 × 3.9 Novapak-phenyl-4
Mobile phase: MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 3.2-3.4

OTHER SUBSTANCES

Simultaneous: trifluoperazine, triflupromazine

REFERENCE

Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee Newguard guard column
Column: 100 × 4.6 5 μm Hypercarb
Mobile phase: MeOH containing 20 mM NaOH
Flow rate: 1
Injection volume: 40
Detector: UV 275

CHROMATOGRAM

Retention time: 9.2

OTHER SUBSTANCES

Simultaneous: related substances

REFERENCE

Karlsson,A.; Berglin,M.; Charron,C. Robustness of the chromatographic separation of alprenolol and related substances using a silica-based stationary phase and selective retention of metoprolol and related substances on a porous graphitic carbon stationary phase, *J.Chromatogr.A*, **1998**, *797*, 75–82.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 7 μm RP-8 guard column
Column: 125 × 4.0 5 μm Hibar LiChrosorb RP-8
Mobile phase: MeCN:pH 3.0 phosphate buffer containing 2 mM sodium *n*-octylsulfonate 29:71
Flow rate: 1
Injection volume: 40
Detector: UV 275

CHROMATOGRAM

Retention time: 8.0

OTHER SUBSTANCES

Simultaneous: related substances

REFERENCE

Karlsson,A.; Berglin,M.; Charron,C. Robustness of the chromatographic separation of alprenolol and related substances using a silica-based stationary phase and selective retention of metoprolol and related substances on a porous graphitic carbon stationary phase, *J.Chromatogr.A*, **1998**, *797*, 75–82.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyram-

ide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 8.28

OTHER SUBSTANCES

Also analyzed: acebutolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-

10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 62:38:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 12.4, 15.9 (enantiomers)
Limit of detection: 31-39 fmole

OTHER SUBSTANCES

Also analyzed: oxprenolol, propranolol

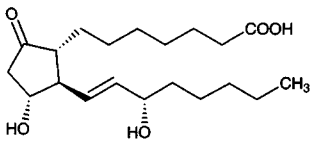
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Alprostadil



Molecular formula: $C_{20}H_{34}O_5$
Molecular weight: 354.49
CAS Registry No.: 745-65-3
Merck Index: 8063
Lednicer No.: 3 2

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Certify C18 SPE cartridge with water, MeCN, and 20 mM citric acid. Add 1 mL plasma to SPE cartridge, wash with 1 mL 20 mM citric acid, wash with 2 mL MeOH:water 10:90, wash with 2 mL cyclohexane, elute with 3 mL 3% ammonia in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeCN, add 200 μL 10 mM DBD-PZ in MeCN, add 300 μL 10 mM 2,2'-dipyridyl disulfide and 10 mM triphenylphosphine in MeCN, let stand at room temperature for 30 min, inject an aliquot. (DBD-PZ prepared from 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN added dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, extract three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ) as orange crystals, mp 121-2° (J. Chromatogr. 1991, 588, 61).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: Gradient. MeCN:water from 35:65 to 60:40 over 1 h
Column temperature: 40
Flow rate: 1
Detector: F ex 440 em 569

CHROMATOGRAM

Retention time: 29.9
Limit of detection: 1.7-5 fmole

OTHER SUBSTANCES

Extracted: dinoprost (prostaglandin F2α), dinoprostone (prostaglandin E2), limaprost, 6-ketoprostaglandin F1α, prostaglandin F1α, prostaglandin D2, prostaglandin A1, prostaglandin B1

KEY WORDS

plasma; rat; SPE

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Terao,T.; Imai,K. Sensitive fluorometric detection of prostaglandins by high performance liquid chromatography after precolumn labelling with 4-(*N,N*-dimethylaminosulphonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ), *Biomed.Chromatogr.*, **1992**, *6*, 143–148.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Serum + 44 μL MeOH + 1 μL pyridine, sonicate for 5 min, add 25 μL 100 mM reagent in DMF, add 20 μL 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, let stand at 25° for 2 h, centrifuge, inject an aliquot. (Reagent

was 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Pass dry hydrogen chloride into a mixture of 12.6 g methyl 2-furoate, 4.5 g paraformaldehyde, and 3.4 g anhydrous zinc chloride in 50 mL dry chloroform for 3 h while holding the reaction temperature at 30°. After cooling pour the contents of the flask into 100 mL cold water, remove the chloroform layer, extract the aqueous layer with chloroform (cf Coll. Czech. Chem. Commun. 1960, 25, 1058). Combine the chloroform layers, neutralize, dry over anhydrous calcium chloride, evaporate, distil to give 5-chloromethyl furyl-2-carboxylic acid methyl ester (bp 108°/4 mm Hg). Reflux 10 g 5-chloromethyl furyl-2-carboxylic acid methyl ester and 25 g silver carbonate in 100 mL THF: water 70:30 for 5 h, filter through Celite, concentrate the filtrate under reduced pressure, chromatograph the product on silica gel with chloroform to give 5-hydroxymethyl furyl-2-carboxylic acid methyl ester as a light yellow oil. Add a solution of 2.9 g 5-hydroxymethyl furyl-2-carboxylic acid methyl ester in 30 mL dichloromethane to 12 g pyridinium chlorochromate in 100 mL, dichloromethane, stir at room temperature for 4 h, evaporate to dryness under reduced pressure, chromatograph on silica with dichloromethane to give 5-formyl furyl-2-carboxylic acid methyl ester as a light yellow powder. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2- nitrophenyl)sulfide as yellow needles (mp 231-232°). Add 15 mL concentrated HCl dropwise to 1.5 g di(4,5-dimethoxy-2-nitrophenyl)sulfide and 4.5 g tin powder stirred at 40-50° in 150 mL EtOH, reflux for 1 h, cool to room temperature, filter, add 1.17 g 5-formyl furyl-2-carboxylic acid methyl ester to the filtrate, reflux for 1 h, cool, filter, chromatograph the solid on silica gel with dichloromethane, recrystallize from EtOH to give 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester as a yellow powder (mp 192-202°). Add 2 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen and may expplode when distilled in air!) to 800 mg 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester in 20 mL EtOH, reflux for 30 min, collect the solid, wash with MeOH, dry under vacuum over phosphorus pentoxide to give 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a light yellow solid (mp 226-228°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 75:25 over 25 min, to 100:0 over 15 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 363 em 452

CHROMATOGRAM

Retention time: 51
Limit of detection: 50 fmole

OTHER SUBSTANCES

Extracted: arachidonic acid, dinoprost, dinoprostone, lauric acid, linoleic acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, prostaglandin $F_{1\alpha}$, stearic acid

KEY WORDS

derivatization

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *Anal.Sci.*, **1995**, *11*, 103–107.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 5-50 mg compound in 1-2 mL MeCN, add a 3-fold molar excess p-nitrophenacyl bromide, add a 2-fold molar excess of N,N-diisopropylethylamine, let stand at room temperature for 15 min, dilute with 50 mL ethyl acetate, wash with 25 mL 200 mM pH 2.30 citrate buffer, wash with 200 mM pH 7.80 phosphate buffer, wash with 25 mL water, dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 45°, prepare a solution in dichloromethane:chloroform 50:50, inject an aliquot.

HPLC VARIABLES

Column: two 250 × 2.1 Zorbax-Sil columns in series
Mobile phase: Dichloromethane:MeCN:DMF 80:20:0.5
Flow rate: 0.28
Detector: UV 254

CHROMATOGRAM

Retention time: 34.25

OTHER SUBSTANCES

Simultaneous: dinoprostone

KEY WORDS

derivatization; normal phase

REFERENCE

Morozowich,W.; Douglas,S.L. Resolution of prostaglandin p-nitrophenacyl esters by liquid chromatography and conditions for rapid, quantitative p-nitrophenacylation, *Prostaglandins*, **1975**, *10*, 19–40.

SAMPLE

Matrix: bulk

Sample preparation: Add 10 mg prostaglandin to 1 mL 15 mg/mL 2-bromo-4'-nitroacetophenone in MeCN, add 5 μL N,N-diisopropylethylamine, mix, let stand at room temperature for at least 2 h. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL chloroform, add 500 μL 200 mg/mL silver nitrate in water, mix thoroughly, centrifuge. Filter (0.2 μm) the chloroform layer and inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil SCX impregnated with silver ion (Prepare the column by pumping 80 mL 1 M silver nitrate in water through the column, wash the column with water until a negative test for silver ion is obtained, wash with 50 mL EtOH, wash with 50 mL acetone, wash with 50 mL ethyl acetate, wash with 50 mL trichloroethane, and wash with 50 mL hexane.)
Mobile phase: Dioxane:MeCN 99.94:0.06 (Caution! Dioxane is a carcinogen!)
Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.2

OTHER SUBSTANCES

Simultaneous: degradation products, dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization

REFERENCE
Merritt,M.V.; Bronson,G.E. High-performance liquid chromatography of p-nitrophenacyl esters of selected prostaglandins on silver ion-loaded microparticulate cation-exchange resin, *Anal.Biochem.*, **1977**, *80*, 392–400.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a 500 μg/mL solution of the bulk drug in EtOH. Evaporate a 2 mL aliquot of the EtOH solution or an aliquot of the formulation containing 1 mg compound to dryness under a stream of nitrogen, add 200 μL 20 mg/mL α-bromoacetonaphthone, swirl, add 100 μL 10 μL/mL N,N-diisopropylethylamine, swirl, heat at 45° for 1 h with swirling every 15 min, evaporate to dryness under a stream of nitrogen, reconstitute with 10 mL 400 μg/mL methylprednisolone in dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μPorasil
Mobile phase: Dichloromethane:1,3-butanediol:water 99.5:0.5:0.05
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 15
Internal standard: methylprednisolone (25)

OTHER SUBSTANCES
Simultaneous: dinoprostone, 8-isoprostaglandin E_1, 8-isoprostaglandin E_2, 5,6-trans-prostaglandin E_2

KEY WORDS
derivatization; injections; normal phase

REFERENCE
Zoutendam,P.H.; Bowman,P.B.; Ryan,T.M.; Rumph,J.L. Quantitative determination of alprostadil (PGE_1) in bulk drug and pharmaceutical formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *283*, 273–280.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 500 μL enzyme incubation to 1 mL MeOH, mix, add 4 mL 100 mM citric acid, add 500 mg anhydrous sodium sulfate, extract twice (alprostadil, dinoprostone) or 3 times (dinoprost) with 5 mL portions of dichloromethane. Pass the extracts through 1 g anhydrous sodium sulfate and evaporate them to dryness, reconstitute with 1 mL anhydrous MeCN containing a 3-fold molar excess of α,p-dibromoacetophenone, add 2 μL diisopropylethylamine, let stand for 1 h, evaporate to dryness, reconstitute with 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Limit of quantitation: 5 μM

OTHER SUBSTANCES
Extracted: metabolites, dinoprost, dinoprostone

KEY WORDS
derivatization

REFERENCE
Fitzpatrick,F.A. High-performance liquid chromatographic analysis of prostaglandins formed during in vitro incubations with prostaglandin 15-dehydrogenase, *J.Pharm.Sci.*, **1976**, *65*, 1609–1613.

SAMPLE
Matrix: formulations
Sample preparation: 100-300 mg Gel ointment + 3 mL MeOH, mix vigorously, filter (0.2 μm). Evaporate 2 mL to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:20 mM pH 4.9 KH_2PO_4 40:60
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Extracted: prostaglandin E1-α-cyclodextrin, prostaglandin A1, prostaglandin B1

KEY WORDS
ointment

REFERENCE
Yamamura,K.; Yamada,J.-I.; Yotsuyanagi,T. High-performance liquid chromatographic assay of antiinflammatory drugs incorporated in gel ointments. Separation and stability testing, *J.Chromatogr.*, **1985**, *331*, 383–388.

SAMPLE
Matrix: seminal fluid
Sample preparation: 1-5 μL Seminal fluid + 100 μL 5 μM IS in MeOH, mix, add 3 mL water to the supernatant, acidify to pH 3-4 with 100 mM HCl, extract with 7 mL ethyl acetate. Remove the ethyl acetate layer and evaporate it to dryness, reconstitute with MeOH. Evaporate to dryness in a clean tube, add 10 mg finely-powdered potassium bicarbonate:sodium sulfate 50:50, add 50 μL 0.4-1 mM 4-bromomethyl-7-acetoxycoumarin in acetone, add 50 μL 200 μM dibenzo-18-crown-6 in acetone, heat in the dark at 80° for 1 h, cool, inject a 20-40 μL aliquot. (Prepare 4-bromomethyl-7-acetoxycoumarin as follows. Reflux 50 g 7-hydroxy-4-methylcoumarin (β-methylumbelliferone) and 100 mL acetic anhydride for 1 h, cool, pour into 500 mL cold water, filter, dry the solid, recrystallize from EtOH to give 4-methyl-7-acetoxycoumarin. Reflux 10 g 4-methyl-7-acetoxycoumarin, 9 g N-bromosuccinimide, a little 2,2'-(azobis(2-methylpropionitrile) (α,α'-azobisisobutyronitrile, Eastman), and 100 mL carbon tetrachloride for 20 h, cool, evaporate under reduced pressure to remove the solvent, wash the residue with water, filter, dry, recrystallize from ethyl acetate/cyclohexane to give 4-bromomethyl-7-acetoxycoumarin (mp 184-185°) (J. Chromatogr. 1982, 234, 121).)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:water from 30:70 to 90:10 over 99 min (Concave 1 curve (64 min) using a Japan Spectroscopic Model GP-A30 solvent programmer).
Column temperature: 50
Flow rate: 1
Injection volume: 20-40
Detector: F ex 365 em 460 following post-column reaction. The effluent from the column mixed with 100 mM NaOH pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 50° to the detector. (The prostaglandins are chromatographed as the coumarin derivatives then hydrolyzed in the post-column reactor to fluorescent 7-hydroxy-4-hydroxymethylcoumarin.)

CHROMATOGRAM
Retention time: 47
Internal standard: 16-methylprostaglandin $F_{1\alpha}$ (49)
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: dinoprost, dinoprostone

KEY WORDS
derivatization; post-column reaction

REFERENCE
Tsuchiya,H.; Hayashi,T.; Naruse,H.; Takagi,N. Sensitive high-performance liquid chromatographic method for prostaglandins using a fluorescence reagent, 4-bromomethyl-7-acetoxycoumarin, *J.Chromatogr.*, **1982**, *231*, 247–254.

SAMPLE
Matrix: seminal fluid
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL EtOH and 20 mL water. Dilute seminal fluid to 1 mL, adjust to pH 3.5 with aqueous formic acid, centrifuge, add the supernatant to the SPE cartridge, wash with 20 mL EtOH:water 15:85, wash with 20 mL water, remove excess water mechanically, wash with 20 mL hexane, elute with 4 mL methyl formate. Dry the eluate under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot (J. Chromatogr. 1985, 349, 431). (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days (J. Chromatogr. 1983, 282, 435).); SPE

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 C18
Mobile phase: Gradient. MeCN:0.5 mM formic acid 30:70 for 4.5 min, to 40:60 (step gradient).
Flow rate: 0.4
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: dinoprostone, 19-hydroxyprostaglandin E_1, 19-hydroxyprostaglandin E_2, oxoprostaglandin E_1, oxoprostaglandin E_2

KEY WORDS
derivatization; SPE

REFERENCE
Doehl,J.; Greibrokk,T. Determination of prostaglandins in human seminal fluid by solid-phase extraction, pyridinium dichromate derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 21–32.

SAMPLE
Matrix: seminal fluid
Sample preparation: Mix 50 μL seminal fluid with 500 μL dilute HCl (pH 3.0) and 500 μL ethyl acetate, vortex. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL water, add to a Toyopak-ODS SPE cartridge, elute with 200 μL MeOH. 100 μL Eluate + 100 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water + 100 μL 1% aqueous pyridine + 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran is as follows. Slowly add 153 g freshly distilled phosphorus oxychloride to 73 g anhydrous DMF with stirring at room temperature, add 125 g sesamol in portions over 4 h, stir at room temperature overnight, pour into ice water, filter. Dissolve the solid in ether and wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as slightly-yellow crystals (mp 125-126°). Pass HCl gas into 15.6 g ethyl 2-thiophenecarboxylate, 4.5 g paraformaldehyde, and 3.4 g zinc chloride in 50 mL chloroform with stirring at 30° over 4 h. Pour into ice water and extract with 50 mL chloroform. Wash the chloroform layer 3 times with water, wash twice with aqueous sodium bicarbonate solution, dry over anhydrous sodium sulfate, evaporate to remove the solvent, distil at 86-94°/0.15 mm Hg to yield ethyl 5-chloromethyl thiophene-2-carboxylate as a colorless oil. Heat 3 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 3.68 g ethyl 5-chloromethyl thiophene-2-carboxylate, and 2.49 g potassium carbonate in 100 mL anhydrous DMF at 110° for 16 h, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with chloroform, recrystallize from chloroform:hexane 25:75 to give 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as yellow crystals (mp 124-126°). Heat 1.5 g 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 1.2 g hydrazine hydrate in 15 mL DMF at 70° for 1 h (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 10 g hydrazine hydrate, add 20 mL water, filter. Wash the solid with MeOH and dry it under reduced pressure to give 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as a yellow powder (mp 262-263°).)

HPLC VARIABLES
Column: 250 × 4 5 μm Wakosil ODS-II 5C18 HG
Mobile phase: MeCN:water 34:66
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 373 em 483

CHROMATOGRAM
Retention time: 125
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization; SPE

REFERENCE
Saito,M.; Ushijima,T.; Sasamoto,K.; Yakata,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran as novel fluorescence derivatization reagents for carboxylic acids in liquid chromatography, *Anal.Chim.Acta*, **1995**, *300*, 243–251.

SAMPLE
Matrix: solutions
Sample preparation: Prepare methyl ester by treatment with excess ethereal diazomethane for 5 min, remove excess reagent under a stream of nitrogen. Dissolve 10 μg methyl ester in 200 μL anhydrous pyridine containing a 10-fold molar excess of p-nitrobenzylhydroxylamine hydrochloride, heat at 40° for 2 h, evaporate to dryness under a stream of nitrogen, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 600 mm long μBondapak C18
Mobile phase: MeCN:water 85:15
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin A1, prostaglandin A2, prostaglandin B1, prostaglandin B2

KEY WORDS
derivatization

REFERENCE
Fitzpatrick,F.A.; Wynalda,M.A.; Kalser,D.G. Oximes for high-performance liquid and electron capture gas chromatography of prostaglandins and thromboxanes, *Anal.Chem.*, **1977**, *49*, 1032–1035.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10 equivalents of reagent, vortex for 1 min, let stand at room temperature for 8 min, add a volume of water equivalent to one tenth the volume of the reaction mixture, inject a 5 μL aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm pellicular C18 (Supelco)
Column: 200 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: Gradient. MeCN:10 mM formic acid from 40:60 to 60:40 over 10 min
Flow rate: 1.5
Injection volume: 5
Detector: UV 228 for 10 min then UV 298

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 30-80 pmole

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin A_1, prostaglandin A_2, prostaglandin B_1, prostaglandin B_2

KEY WORDS
derivatization

REFERENCE
Dohl,J.; Greibrokk,T. High-performance liquid chromatographic separation and ultraviolet detection of prostaglandins, oxidized by pyridinium dichromate, *J.Chromatogr.*, **1983**, *282*, 435–442.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, inject a 1 μL aliquot. Alternatively, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Column: 250 × 1.3 8 μm C18 (Chrompack)
Mobile phase: MeCN:10 mM pH 2.7 phosphoric acid 38:62
Flow rate: 0.06
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 28
Limit of detection: 0.14 pmole

OTHER SUBSTANCES
Simultaneous: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization; microbore

REFERENCE
Doehl,J.; Greibrokk,T. High-performance liquid chromatographic separation and determination of prostaglandins, oxidized by pyridinium dichromate. Optimization and applications, *J.Chromatogr.*, **1985**, *349*, 431–438.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of a solution in MeOH with 50 μL purified 9-anthryldiazomethane reagent, after 6 h inject an aliquot. (Purify 9-anthryldiazomethane on a 500 × 7.2 7 μm PG-pak C polystyrene gel column with ethyl acetate at 1 mL/min and UV 350 detection, inject 1 mg, collect the effluent when the purified compound elutes (20-22 min) and use it within 6 h.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm PG-Pak B silica gel
Mobile phase: Gradient. Isooctane:ethyl acetate:EtOH:acetic acid 90:10:0:1 for 15 min then 80:15:4:2 for 20 min (step gradient).
Flow rate: 1.2
Detector: F ex 365 em 412

CHROMATOGRAM
Retention time: 32.5
Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: dinoprost, dinoprostone, HHT, hydroxyeicosatetraenoic acid, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, prostaglandin $F_{1\alpha}$, thromboxane B_2

KEY WORDS
derivatization; normal phase

REFERENCE
Yamauchi,Y.; Tomita,T.; Senda,M.; Hirai,A.; Terano,T.; Tamura,Y.; Yoshida,S. High-performance liquid chromatographic analysis of arachidonic acid metabolites by pre-column derivatization using 9-anthryldiazomethane, *J.Chromatogr.*, **1986**, *357*, 199–205.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 5 μm cyano (IBM)
Mobile phase: Gradient. Hexane:isopropanol 98:2 for 12 min, then to 80:20 over 10 min, maintain at 80:20
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: k' 10.54

OTHER SUBSTANCES
Extracted: arachidonic acid, prostaglandin H2, dinoprostone (prostaglandin E2), prostaglandin D2, dinoprost (prostaglandin F2α), prostaglandin F1α, 6-ketoprostaglandin E1, 6-ketoprostaglandin F1α, thromboxane B2

REFERENCE
Zulak,I.M.; Puttemans,M.L.; Schilling,A.B.; Hall,E.R.; Venton,D.L. A fast, nondestructive purification scheme for prostaglandin H2 using a nonaqueous, bonded-phase high-performance liquid chromatography system, *Anal.Biochem.*, **1986**, *154*, 152–161.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compound in 1 mL MeCN:THF 80:20, add 70 μg panacyl bromide, add 1.025 μL N,N-diisopropylethylamine, mix, let stand at room temperature for 3 h, inject an aliquot onto column A (pre-equilibrated with 10 mL dichloromethane) and elute to waste with 15 mL dichloromethane, elute the contents of column A onto column B with the mobile phase and start the gradient, monitor the effluent from column B. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80,

add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°).)

HPLC VARIABLES

Column: A Guard-Pak silica; B 250 × 4.6 5 μm Hibar Silica (Merck)
Mobile phase: Gradient. A was hexane:dichloromethane:THF:MeCN:MeOH 35:50:11:4:0.25. B was dichloromethane:MeOH 98:2. C was dichloromethane:MeOH:THF 92:7:1. A:B:C 100:0:0 for 35 min, to 0:100:0 over 10 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 15 min
Flow rate: 1
Injection volume: 20
Detector: F ex 253 em 445

CHROMATOGRAM

Retention time: 71
Limit of detection: 30 pg

OTHER SUBSTANCES

Simultaneous: 13,14-dihydro-15-ketoprostaglandin E_2, dinoprost, dinoprostone, 11-epi-prostaglandin E_2, 8-isoprostaglandin E_2, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin A_2, prostaglandin D_2, thromboxane B_2

KEY WORDS

derivatization; column-switching; normal phase

REFERENCE

Salari,H.; Yeung,M.; Douglas,S.; Morozowich,W. Detection of prostaglandins by high-performance liquid chromatography after conversion to *p*-(9-anthroyloxy)phenacyl esters, *Anal.Biochem.*, **1987**, *165*, 220–229.

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 μL of a 0.01-10 μg/mL solution in MeOH with 100 μL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, let stand at room temperature for 1.5 h, inject a 5 μL aliquot. (Synthesis of 1-pyrenyldiazomethane is as follows. Suspend 5 g 1-pyrenecarboxaldehyde in 80 mL EtOH, add 3.4 g hydrazine monohydrate (Caution! Hydrazine monohydrate is a carcinogen!), stir at room temperature for 3 h, filter off the product and wash it with 50 mL cold EtOH, recrystallize from EtOH to obtain 1-pyrenecarboxaldehyde hydrazone as yellow crystals (mp 186-194° d). Add 6.55 g activated manganese dioxide to 2 g 1-pyrenecarboxaldehyde hydrazone in 300 mL diethyl ether, sonicate at room temperature for about 80 min (monitor by HPLC), filter, wash the solid with a little ether, evaporate the filtrate to obtain 1-pyrenyldiazomethane as red crystals. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971). 1-Pyrenyldiazomethane is also available from Molecular Probes, Eugene OR.)

HPLC VARIABLES

Column: 150 × 4 5 μm TSK-GEL-120A ODS (TOSOH)
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 5
Detector: F ex 340 em 395

CHROMATOGRAM

Retention time: 14

Limit of detection: 20-30 fmole

OTHER SUBSTANCES

Simultaneous: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization

REFERENCE

Nimura,N.; Kinoshita,T.; Yoshida,T.; Uetake,A.; Nakai,C. 1-Pyrenyldiazomethane as a fluorescent labeling reagent for liquid chromatographic determination of carboxylic acids, *Anal.Chem.*, **1988**, *60*, 2067–2070.

SAMPLE

Matrix: solutions

Sample preparation: Mix 200 μL of a 10 μM solution in DMF containing 140 mM diethylphosphorocyanidate with 200 μL 10 mM DBD-PZ in MeCN, let stand at room temperature for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water,

dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 45:55
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 15
Limit of detection: 14 fmol

OTHER SUBSTANCES

Simultaneous: dinoprost, hydrocortisone succinate, prednisolone succinate

KEY WORDS

SPE

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 100 μM solution of the carboxylic acid in water with 100 μL 100 mM 1-(3-methylaminopropyl)-3-ethylcarbodiimide in water, 100 μL 1% pyridine in water, and 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and

dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°) (Anal. Sci. 1989, 5, 525). 2-(5-Chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is also available from Dojindo, Kumamoto, Japan. Add 2 mL hydrazine hydrate to a stirred solution of 2 g 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in 20 mL anhydrous DMF (Caution! Hydrazine hydrate is a carcinogen!), stir at room temperature for 4 h, add 20 mL benzene (Caution! Benzene is a carcinogen!). Collect the precipitate and wash it with water and MeCN, recrystallize from DMF:benzene 50:50 to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as an off-white crystalline solid (mp >220° d).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil ODS-II, WS-II 5C18 HG
Mobile phase: MeCN:water 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 450

CHROMATOGRAM

Retention time: 76
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Simultaneous: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization

REFERENCE

Saito,M.; Chiyoda,Y.; Ushijima,T.; Sasamoto,K.; Ohkura,Y. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *Anal.Sci.*, **1994**, *10*, 679–681.

SAMPLE

Matrix: solutions
Sample preparation: 100 μL 10 mM compound in MeOH + 100 μL 1% pyridine in MeOH + 100 μL 15 mM reagent in DMSO, 100 μL 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, heat at 37° for 1 h, inject a 10 μL aliquot. (Reagent was 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2-nitrophenyl)sulfide as yellow needles (mp 231-232°) (Anal. Sci. 1995, 11, 103). Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Reflux 5.0 g ethyl 2-chloromethyl-5-oxazolecarboxylate and 11.7 g NaI in 80 mL acetone for 1 h, partition the

reaction mixture between ethyl acetate and water. Wash the organic layer with water and dry it over anhydrous sodium sulfate, evaporate to give ethyl 2-iodomethyl-5-oxazolecarboxylate as a reddish-brown oil. Reflux 7.4 g ethyl 2-iodomethyl-5-oxazolecarboxylate and 21.5 g silver carbonate in 100 mL THF:water 70:30 for 4 h, filter through Celite, evaporate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-hydroxymethyl-5-oxazolecarboxylate (mp 60.5-62°). Stir 2.04 g oxalyl chloride in 15 mL dichloromethane at -50° under nitrogen, add 1.54 g DMSO in 3 mL dichloromethane, after 5 min add 1.4 g ethyl 2-hydroxymethyl-5-oxazolecarboxylate in 6 mL dichloromethane, stir for 15 min at -50°, add 5.7 mL triethylamine, allow to warm to room temperature, dilute with dichloromethane, wash with water, dry over anhydrous sodium sulfate, concentrate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-carboxaldehyde-5-oxazolecarboxylate (mp 71.5-73°). Add 11.3 mL concentrated HCl to 750 mg di(4,5-dimethoxy-2-nitrophenyl)sulfide stirred in 100 mL EtOH, add 3.3 g tin powder at 40-45°, stir for 1 h at 40-45°, dilute with 100 mL water, pass hydrogen sulfide gas through this solution (Caution! Hydrogen sulfide is highly toxic!), filter, concentrate the filtrate under reduced pressure to give 4,5-dimethoxy-2-aminothiophenol. Take up this compound in 30 mL EtOH:acetic acid 2:1 and add 750 mg ethyl 2-carboxaldehyde-5-oxazolecarboxylate, reflux for 1 h, collect the precipitate and recrystallize it from EtOH to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as yellow needles (mp 200-201°). Add 381 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole to 20 mL EtOH containing 3 mL DMF and 5 mL hydrazine hydrate, reflux for 1 h, collect the precipitate and wash it with EtOH, dry under vacuum to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a yellow powder (mp 255.5-280° (d)).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 20 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 369 em 451

CHROMATOGRAM

Retention time: 38.5

OTHER SUBSTANCES

Simultaneous: dinoprost, dinoprostone, prostaglandin F1α

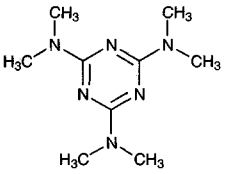
KEY WORDS

derivatization

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *J.Chromatogr.B*, **1995**, *674*, 167–175.

Altretamine



Molecular formula: $C_9H_{18}N_6$
Molecular weight: 210.28
CAS Registry No.: 645-05-6
Merck Index: 328

SAMPLE

Matrix: blood
Sample preparation: Cool serum or plasma and MeCN to 4°. Slowly add 600 μL MeCN to 200 μL serum or plasma, vortex for 10 s, filter (Sartorius RC4 syringe filter), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 mm long Alltech C18
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:buffer 70:30 (Buffer was 3.14 g $NaH_2PO_4.12H_2O$ and 0.108 g $Na_2HPO_4.2H_2O$ in 1 L water, pH 8.2.)
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 8.5
Limit of quantitation: 150 ng/mL

OTHER SUBSTANCES

Extracted: pentamethylmelamine, 2,2,4,6-tetramethylmelamine, metabolites

KEY WORDS

serum; plasma; pharmacokinetics

REFERENCE

Barker,I.K.; Crawford,S.M.; Fell,A.F. Determination of altretamine in human plasma with high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *660*, 121–126.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 229.9

CHROMATOGRAM
Retention time: 17.833

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2×10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

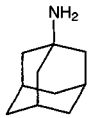
CHROMATOGRAM
Retention time: 6.2
Internal standard: altretamine
Limit of detection: 2 pmol
Limit of quantitation: 8 pmol

OTHER SUBSTANCES
Extracted: daunorubicin, doxorubicin, verapamil, vincristine, S 9788

KEY WORDS
human; cells; epidermoid carcinoma; altretamine is IS

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

Amantadine



Molecular formula: $C_{10}H_{17}N$
Molecular weight: 151.25
CAS Registry No.: 768-94-5, 665-66-7 (HCl)
Merck Index: 389
Lednicer No.: 2 18

SAMPLE

Matrix: blood, urine

Sample preparation: Add NaOH to plasma and urine so that the final NaOH concentration is 50 mM. Wash column A with 400 μL MeCN, with 400 μL 1 mM sodium dodecyl sulfate in MeCN:water 40:60, and with 400 μL 5 mM sodium dodecyl sulfate in water. Inject a 50 μL aliquot of plasma or urine at 0.36 mL/min, inject 50 μL 5 mM sodium dodecyl sulfate in water at 0.36 mL/min, after 3 min wash column A with 400 μL 5 mM sodium dodecyl sulfate in water, backflush the contents of column A onto column B with mobile phase and start the gradient, after 15 s remove column A from the circuit and wash it with 400 μL MeCN, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 27 × 2.1 polymeric reagent; B reverse-phase (not otherwise specified) (Prepare polymeric reagent as follows. Prepare a porous rigid resin using a divinylbenzene:ethylstyrene:styrene 24:6:70 mixture with trimethylsilyl modified silica (102 Å average pore size, 1.08 mL/g pore volume, 366 m^2/g surface area, 16-20 μm irregular particle shape, IMPAQ RG 1020 Si silica, PQ Co., Valley Forge PA). Further preparation details are not given but a typical procedure given in the cited reference is as follows. Aerate a mixture of 10 g modified silica in 100 mL water with nitrogen for 15 min, add 10 mL styrene:80% divinylbenzene:t-butyl peroxybenzoate 49:49:2 (remove preservative by passing through a butylcatechol remover (Scientific Polymer, Ontario NY), shake vigorously at room temperature for 4 h, add 150 mL 0.75% polyvinyl alcohol, shake for 4 h, heat at 120° for 24 h while shaking on a Parr instrument, cool to room temperature, filter, wash with 100 mL water, wash with 50 mL MeOH. Add the solid to 500 mL 3 M NaOH in MeOH:water 40:60, shake at room temperature for 14 h (to dissolve the silica), filter, wash with water until the washings are neutral, wash with 100 mL MeOH, dry at 60°. The polymer has similar properties to the template silica (US Pat. 4 933 372 (1990)). Soxhlet extract the resin with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

Mobile phase: Gradient. MeCN:water 55:45 for 1.5 min, to 85:15 over 4 min, maintain at 85:15 for 5 min, return to initial conditions over 1 min, re-equilibrate for 2 min.
Column temperature: 75 (column A only)
Flow rate: 1.5
Injection volume: 50
Detector: F ex 254 em 305-395

CHROMATOGRAM

Retention time: 8.7
Limit of detection: 0.74 ng (urine), 0.79 ng (plasma)

KEY WORDS

derivatization; plasma; column-switching

REFERENCE

Zhou,F.-X.; Krull,I.S.; Feibush,B. Direct determination of adamantanamine in plasma and urine with automated solid phase derivatization, *J.Chromatogr.*, **1993**, *619*, 93–101.

SAMPLE

Matrix: solutions
Sample preparation: Wash 70 mg polymeric reagent with 500 μL MeCN, heat at 75°, inject 50 μL of an amantadine solution in 50 mM NaOH, let stand at 75° for 3 min, flush contents of the reactor onto the column with mobile phase. Flush reactor with MeCN between runs. (Prepare polymeric reagent as follows. Prepare a porous rigid resin using a divinylbenzene:ethylstyrene:styrene 24:6:70 mixture with trimethylsilyl modified silica (102 Å average pore size, 1.08 mL/g pore volume, 366 m^2/g surface area, 16-20 μm irregular particle shape, IMPAQ RG 1020 Si silica, PQ Co., Valley Forge PA). Further preparation details are not given but a typical procedure given in the cited reference is as follows. Aerate a mixture of 10 g modified silica in 100 mL water with nitrogen for 15 min, add 10 mL styrene:80% divinylbenzene:t-butyl peroxybenzoate 49:49:2 (remove preservative by passing through a butylcatechol remover (Scientific Polymer, Ontario NY), shake vigorously at room temperature for 4 h, add 150 mL 0.75% polyvinyl alcohol, shake for 4 h, heat at 120° for 24 h while shaking on a Parr instrument, cool to room temperature, filter, wash with 100 mL water, wash with 50 mL MeOH. Add the solid to 500 mL 3 M NaOH in MeOH:water 40:60, shake at room temperature for 14 h (to dissolve the silica), filter, wash with water until the washings are neutral, wash with 100 mL MeOH, dry at 60°. The polymer has similar properties to the template silica (US Pat. 4 933 372 (1990)). Soxhlet extract the resin with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak C18
Mobile phase: MeCN:water 65:35
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: octylamine

KEY WORDS
derivatization

REFERENCE
Szulc,M.; Swett,P.; Krull,I.S. Size-selective derivatizations with polymer immobilized reagents, *Biomed.Chromatogr.*, **1997**, *11*, 207–223.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 200 μL 50 nM n-decylamine in benzene + 100 μL 1 M NaOH (Caution! Benzene is a carcinogen!), shake mechanically for 15 min, centrifuge at 6400 rpm for 6 min. Remove a 20 μL aliquot of the organic layer and add it to 10 μL 250 μM 3-(4,6-difluorotriazinyl)amino-7-methoxycoumarin in benzene, heat at 140° for 15 min, cool to room temperature, inject a 10 μL aliquot. (Synthesis of 3-(4,6-difluorotriazinyl)amino-7-methoxycoumarin is as follows. Heat 2-hydroxy-4-methoxybenzaldehyde with 0.5 equivalents glycine, 1.25 equivalents fused sodium acetate, and 5 parts freshly distilled acetic anhydride at 140° for 1 h and at 160° for 1 h, cool to 100°, pour into water containing cracked ice, let stand overnight. Wash the solid with very dilute NaOH, cold water, and a few mL of hot MeOH. Crystallize from acetic acid to obtain 3-acetamido-7-methoxycoumarin (mp 230°). Suspend 1 g 3-acetamido-7-methoxycoumarin in 25 mL acetic acid and 25 mL 50% sulfuric acid, heat at 50-60° for 30-45 min, pour into an equal volume of cold water, neutralize with sodium bicarbonate, recrystallize from EtOH/water to obtain 3-amino-7-methoxycoumarin (mp 154°) (J. Ind. Chem. Soc. 1971, 48, 371). Stir 300 mg 3-amino-7-methoxycoumarin in 100 mL anhydrous ether, slowly add 1 mL cyanuric fluoride (Fluka), stir at room temperature for 1 h, evaporate to dryness under reduced pressure, recrystallize from n-hexane to give 3-(4,6-difluorotriazinyl)amino-7-methoxycoumarin as colorless prisms (mp 212-215°). 3-(4,6-Difluorotriazinyl)amino-7-methoxycoumarin reacts with primary and secondary alkyl amines but not with aromatic amines or alcohols.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK gel ODS-120T (Tosoh)
Mobile phase: MeCN:water 50:10
Flow rate: 1
Injection volume: 10
Detector: F ex 345 em 410

CHROMATOGRAM
Retention time: 14
Internal standard: n-decylamine (26)
Limit of detection: 2.5 nM

KEY WORDS
derivatization

REFERENCE

Fujino,H.; Goya,S. A fluorogenic reagent, 3-(4,6-difluorotriazinyl)amino-7-methoxycoumarin, for the determination of amantadine by high-performance liquid chromatography, *Chem.Pharm.Bull.*, **1990**, *38*, 544–545.

SAMPLE

Matrix: urine

Sample preparation: Prepare a derivatization vial by adding 160 μL 300 mM cetyltrimethylammonium bromide in MeOH to a vial (Eppendorf no. 3813) and evaporating the MeOH at room temperature. 5 mL Urine adjusted to about pH 11 with 10 M NaOH, centrifuge at 3000 g for 10 min. Add 450 μL supernatant and 50 μL 400 mM pH 11 sodium borate buffer to a derivatization vial, vortex for 15 s, add 25 μL 800 mM 2,4-dinitrofluorobenzene in acetone, mix. Remove a 100 μL aliquot and allow it to pass back and forth through a 100 mm length of 0.5 mm i.d. PTFE tubing at 60° for 4 min, inject a 25 μL aliquot. Backflush PTFE tubing with 1 mL MeCN and 1 mL water.

HPLC VARIABLES

Column: 150 × 3 5 μm Hypersil ODS

Mobile phase: MeCN:10 mM pH 2.5 citrate buffer 75:25 containing 20 mM tetramethylammonium bromide

Injection volume: 25

Detector: UV 350

CHROMATOGRAM

Retention time: 5

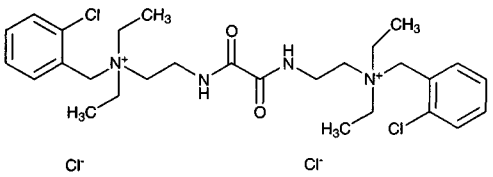
Limit of detection: 75 ng/mL

REFERENCE

van der Horst,F.A.L.; Teeuwsen,J.; Holthuis,J.J.M.; Brinkman,U.A.T. High-performance liquid chromatographic determination of amantadine in urine after micelle-mediated pre-column derivatization with 1-fluoro-2,4-dinitrobenzene, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 799–804.

Ambenonium chloride

Molecular formula: $C_{28}H_{42}Cl_4N_4O_2$
Molecular weight: 608.48
CAS Registry No.: 115-79-7, 52022-31-8 (tetrahydrate)
Merck Index: 396



SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut CBA (weak cation-exchange resin) SPE cartridge with 1 mL 200 mM pH 6.0 sodium phosphate buffer, two 1 mL portions of water, 1 mL MeCN, two 1 mL portions of water, two 1 mL portions of 100 mM HCl, three 1 mL portions of water, and two 1 mL portions of 200 mM pH 6.0 sodium phosphate buffer. 1 mL Serum + 500 μL 0.2 μg/mL timepidium bromide in water + 40 mL 200 mM pH 6.0 sodium phosphate buffer, pass through the SPE cartridge at 2.5 mL/min, wash with three 5 mL portions of 200 mM pH 6.0 sodium phosphate buffer, wash with three 1 mL portions of water, wash with 500 μL MeCN:water 50:50, wash with 1 mL water, elute with 250 μL MeCN:2 M lithium perchlorate 50:50, inject a 200 μL aliquot of the eluate. (Siliconize all apparatus or use PTFE containers.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeCN:100 mM pH 3.5 ammonium chloride buffer containing 25 mM lithium perchlorate 30:70
Column temperature: 25
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 8.5
Internal standard: timepidium bromide (21)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Simultaneous: methylbenactyzium bromide
Noninterfering: oxapium iodide, domiphen bromide, propantheline bromide, clocapramine hydrochloride, benzethonium chloride, benzalkonium chloride, neostigmine bromide, distigmine bromide, pralidoxime iodide, prednisolone, atropine

KEY WORDS

serum; SPE; pharmacokinetics

REFERENCE

Ohtsubo,K.; Higuchi,S.; Aoyama,T.; Fujii,N.; Goto,I. Sensitive determination of ambenonium chloride in serum from patients with myasthenia gravis using ion-exchange resin extraction and reversed-phase ion-pair chromatography, *J.Chromatogr.*, **1989**, *496*, 397–406.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g

for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 10.543

KEY WORDS

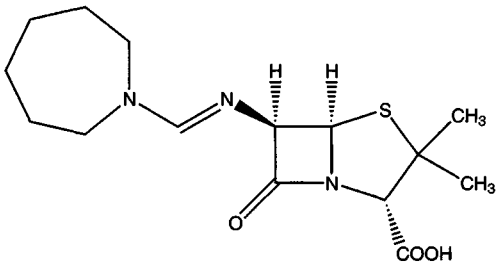
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Amdinocillin

Molecular formula: $C_{15}H_{23}N_3O_3S$
Molecular weight: 325.43
CAS Registry No.: 32887-01-7
Merck Index: 408



SAMPLE

Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:50 mM pH 4.6 KH_2PO_4 buffer 14:86
Flow rate: 1
Injection volume: 100
Detector: UV 218

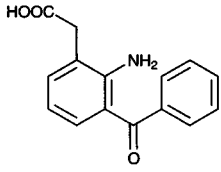
CHROMATOGRAM

Retention time: 5.3
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE

Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

Amfenac



Molecular formula: $C_{15}H_{13}NO_3$
Molecular weight: 255.27
CAS Registry No.: 51579-82-9, 61618-27-7 (sodium salt monohydrate)
Merck Index: 413
Lednicer No.: 3 38

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL water + 200 mg ammonium sulfate + 100 μL 100 μg/mL fenbufen in EtOH, vortex for 30 s, centrifuge at 6000 g for 5 min, filter (0.22 μm) the supernatant, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Bensil-ODS (Bentec)
Mobile phase: Gradient. A was MeCN:50 mM pH 7.0 potassium phosphate buffer 20:80. B was MeCN. A:B 100:0 for 10 min then to 20:80 over 20 min.
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 5.2
Internal standard: fenbufen (8.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

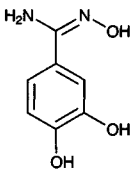
KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kato,Y.; Shimokawa,M.; Yokoyama,T.; Mohri,K. Simultaneous determination of amfenac sodium and its metabolite (7-benzoyl-2-oxindole) in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *616*, 67–71.

Amidox



Molecular formula: $C_7H_8N_2O_3$
Molecular weight: 168.15
CAS Registry No.: 95933-72-5, 95933-73-6 (HCl)

SAMPLE

Matrix: bile, blood

Sample preparation: Bile. Add 90 μL 10 mM pH 6.6 ammonium acetate and 100 μL saturated NaCl in water to 10 μL bile. Extract twice with 1 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen. Dissolve the residue in 200 μL MeOH:water 50:50 and inject a 100 μL aliquot. Plasma. Add 100 μL saturated NaCl in water to 100 μL plasma and extract twice with 1 mL ethyl acetate by vortexing. Combine the organic layers and evaporate them to dryness under a stream of nitrogen. Dissolve the residue in 200 μL MeOH:water 50:50 and inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm LiChrospher RP 18
Column: 250 × 4.6 5 μm LiChrospher RP 18
Mobile phase: Gradient. A was 50 mM potassium phosphate containing 5 mM heptanesulfonic acid, adjusted to pH 4.0 with phosphoric acid. B was MeOH. A:B from 100:0 to 92:8 over 15 min, to70:30 over 15 min, to 60:40 over 2 min, maintain at 60:40 for 10 min, to 100:0 over 2 min, re-equilibrate for 15 min.
Flow rate: 1
Injection volume: 100
Detector: UV 264

CHROMATOGRAM

Retention time: 28.4
Limit of detection: 60 ng/mL (bile), 85 ng/mL (plasma)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; bile; plasma; pharmacokinetics

REFERENCE

Vielnascher,E.; Romanová,D.; Novotny,L.; Szekeres,T.; Elfort,H.L.; Thalhammer,T.; Jäger,W. Simultaneous determination of the new anticancer agent amidox and its metabolites in rat bile and plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *696*, 267–274.

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 0.1 mM solution in 10 mM KH_2PO_4, adjust pH to 6 with a few drops 5 M KOH or phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Supelcosil LC18
Mobile phase: MeOH:buffer 5:95 (Buffer was 0.05% triethylamine adjusted to pH 6 with 50 mM phosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 255

OTHER SUBSTANCES

Simultaneous: didox, trimidox

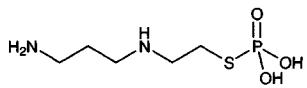
KEY WORDS

comparison with DC polarography and UV spectrophotometry

REFERENCE

Romanova,D.; Vachalkova,A.; Szekeres,T.; Elford,H.L.; Novotny,L. The new inhibitors of ribonucleotide reductase -comparison of some physico-chemical properties, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 951–956.

Amifostine



Molecular formula: $C_5H_{15}N_2O_3PS$
Molecular weight: 214.23
CAS Registry No.: 20537-88-6, 63717-27-1 (monohydrate), 112901-68-5 (trihydrate)
Merck Index: 424
Lednicer No.: 5 1

SAMPLE

Matrix: blood
Sample preparation: 90 μL Plasma + 50 μL 1.4 μg/mL IS + 160 μL 50 mM pH 10 sodium borate/KCl buffer, add 200 μL 5 mg/mL fluorescamine in acetone (dried over 4 Å molecular sieve) while vortexing, vortex for 1 min, add 200 μL 5 mg/mL fluorescamine in acetone (dried over 4 Å molecular sieve), vortex for 20-30 s, centrifuge at 1500 rpm for 3 min, inject a 20-50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: CoPell C18
Column: 100 × 8 5 μm spherical C18 RCM-100 (Waters)
Mobile phase: MeCN:water 22:78 containing 10 mM dibutylammonium phosphate, pH 3 (Prepare solutions of dibutylamine phosphate by adjusting pH of dibutylamine solution to 2.5 with phosphoric acid. Flush column with MeOH:water 70:30 at the end of each day.)
Flow rate: 2
Injection volume: 20-50
Detector: F ex 395 em >460

CHROMATOGRAM

Retention time: 13.1
Internal standard: ^{14}C-labeled amifostine (Collect fraction containing amifostine and count it.)
Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

derivatization; plasma; dog; pharmacokinetics

REFERENCE

Swynnerton,N.F.; McGovern,E.P.; Mangold,D.J.; Niño,J.A.; Gause,E.M.; Fleckenstein,L. HPLC assay for S-2-(3-aminopropylamino)ethyl phosphorothioate (WR 2721) in plasma, *J.Liq.Chromatogr.*, **1983**, *6*, 1523–1534.

SAMPLE

Matrix: blood
Sample preparation: 150 μL Plasma + 150 μL IS in 50 mM pH 10 sodium borate/KCl buffer + 200 μL 50 mM pH 7.6 sodium borate/KCl buffer, vortex, add 250 μL reagent, mix for 1 min, add 250 μL reagent, mix for 20-30 s, centrifuge at 1500 rpm for 3 min. Filter (0.45 μm) the supernatant and inject an aliquot of the supernatant. (Reagent was 5 mg/mL fluorescamine in acetone that had been previously dried over 4Å molecular sieve. Prepare fresh each week.)

HPLC VARIABLES

Guard column: CoPell C18

Column: 100 × 8 5 μm spherical C18 RCM-100 radial compression module (Waters)
Mobile phase: MeCN:water 22:78 containing 10 mM tetrabutylammonium phosphate, pH 3 or MeCN:EtOH:water 16:7:77 containing 10 mM tetrabutylammonium phosphate, pH 3 (Prepare 1 M solutions of tetrabutylammonium phosphate by adjusting the pH of 40% tetrabutylammonium hydroxide to pH 2.5 with phosphoric acid and adjusting to an appropriate volume.)
Flow rate: 2
Injection volume: 50
Detector: F ex 370 (bandpass filter) em 418-700 (cutoff filter)

CHROMATOGRAM

Internal standard: S-3-(4-aminobutylamino)propyl phosphorothioate (WR 80855)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

derivatization; plasma; dog; pharmacokinetics

REFERENCE

Swynnerton,N.F.; McGovern,E.P.; Niño,J.A.; Mangold,D.J. An improved HPLC assay for *S*-2-(3-aminopropylamino)ethyl phosphorothioate (WR-2721) in plasma, *Int.J.Radiat.Oncol.Biol.Phys.*, **1984**, *10*, 1521–1524.

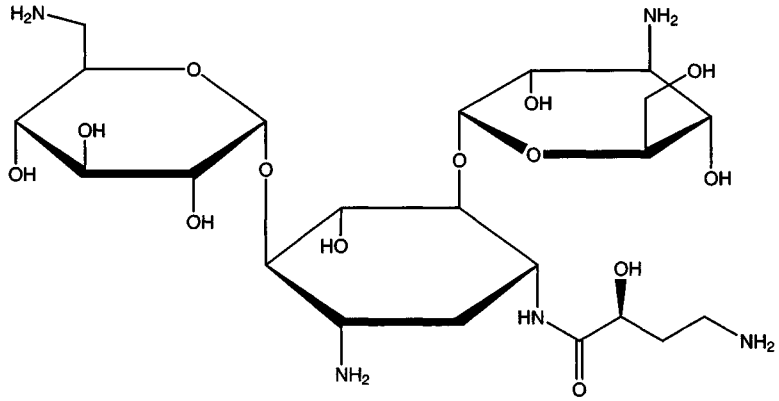
Amikacin

Molecular formula: $C_{22}H_{43}N_5O_{13}$

Molecular weight: 585.61

CAS Registry No.: 37517-28-5, 39831-55-5 (sulfate)

Merck Index: 425



SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water. 50 μL Serum + 25 μL Tris buffer + 100 μL 16 μg/mL kanamycin in MeCN, vortex, centrifuge at 15000 g for 1 min. Remove the supernatant and add it to 30 μL 250 mg/mL 2,4,6-trinitrobenzene-1-sulfonic acid in MeCN, vortex, heat at 70° for 30 min. Add 700 μL wash solution then 250 μL sample solution to the SPE cartridge, wash with 3 column volumes of wash solution, elute with 300 μL MeCN, mix the eluate, inject a 50 μL aliquot of the eluate. (Tris buffer was 2 M pH 10.3 and was prepared by dissolving 24.2 g Trizma base in 100 mL water. Wash solution (10 mM) was 1.82 g potassium hydrogen phosphate in 1 L water adjusted to pH 8.6 with phosphoric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere octyl

Mobile phase: MeCN:20 mM buffer 52:48 (Buffer was 2.68 g KH_2PO_4 in 1 L water adjusted to pH 3.0 with phosphoric acid.)

Column temperature: 50

Flow rate: 2

Injection volume: 50

Detector: UV 340

CHROMATOGRAM

Retention time: 8

Internal standard: kanamycin (12)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, acetazolamide, N-acetylprocainamide, amobarbital, ampicillin, amitriptyline, caffeine, cefamandole, cefoxime, cefoxitin, cephalothin, clindamycin, chloramphenicol, chlordiazepoxide, diazepam, erythromycin, ethosuximide, gentamicin, nitrofurantoin, penicillin G, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, secobarbital, tetracycline, theophylline, tobramycin, vancomycin

KEY WORDS
serum; SPE

REFERENCE
Kabra,P.M.; Bhatnager,P.K.; Nelson,M.A. Liquid chromatographic determination of amikacin in serum with spectrophotometric detection, *J.Chromatogr.*, **1984**, *307*, 224–229.

SAMPLE
Matrix: blood, urine
Sample preparation: Place 400 μL plasma in an Amicon Centrifree Micropartition system unit and centrifuge at 800 g for 50 min. Mix a 200 μL aliquot of plasma ultrafiltrate or urine with 200 μL 1% tris(hydroxymethyl)aminomethane (TRIS) in water, 400 μL DMSO, and 400 μL 1.5% 1-fluoro-2,4-dinitrofluorobenzene in 95% EtOH. Heat at 55° for 30 min with mixing at 5 min intervals. Cool at room temperature for 30 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 37-50 μm C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:2-methoxyethanol:THF:glacial acetic acid:1% tris(hydroxymethyl) aminomethane (TRIS) in water 41:4.52:4.24:0.21:50, pH 7.00 ± 0.02
Column temperature: 58
Flow rate: 1 (0-8.5 min), 0.5 (8.5-19 min), 3 (19-25 min), 1 (25-30 min)
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 16.5
Limit of quantitation: 10000 ng/mL (urine), 500 ng/mL (plasma)

OTHER SUBSTANCES
Noninterfering: cefepime

KEY WORDS
plasma

REFERENCE
Papp,E.A.; Knupp,C.A.; Barbhaiya,R.H. High-performance liquid chromatographic assays for the quantification of amikacin in human plasma and urine, *J.Chromatogr.*, **1992**, *574*, 93–99.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injection to 1 mg/mL with water. Mix 125 μL diluted injection with 1.60 mL pyridine, 500 μL 0.9% (w/v) 4-dimethylaminopyridine solution, and 500 μL 0.5% (w/v) 2,4,6-trinitrobenzenesulfonic acid reagent, shake, heat at 75°. for 75 min. Let the solution to cool to room temperature, then add 500 μL trifluoroacetic acid:MeCN 20:80, shake, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Zorbax SB-C8
Mobile phase: MeCN:MeOH:20 mM monobasic potassium phosphate 41:14:45 adjusted to pH 7.7 with 50% NaOH
Flow rate: 2.0
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 9.7

KEY WORDS
injections; derivatization.

REFERENCE
Rick Lung,K.; Kassal,K.R.; Green,J.S.; Hovsepian,P.K. Catalytic precolumn derivatization of amikacin, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 905–910.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: C18
Mobile phase: MeOH:buffer 72:28 (Buffer was 0.27% KH_2PO_4 adjusted to pH 6.5 with 400 mM KOH.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 340

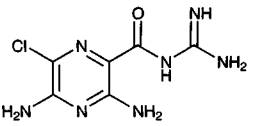
CHROMATOGRAM
Retention time: 7.6

OTHER SUBSTANCES
Simultaneous: cefpirome

KEY WORDS
stability-indicating

REFERENCE
Allen,L.V.,Jr.; Stiles,M.L.; Prince,S.J.; Sylvestri,M.F. Stability of cefpirome sulfate in the presence of commonly used intensive care drugs during simulated Y-site injection, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2427–2433.

Amiloride



Molecular formula: $C_6H_8ClN_7O$
Molecular weight: 229.63
CAS Registry No.: 2609-46-3, 2016-88-8 (HCl), 17440-83-4 (HCl dihydrate)
Merck Index: 426
Lednicer No.: 1 278

SAMPLE

Matrix: blood
Sample preparation: Add 50 μL 1 μg/mL prazosin in MeOH to 1 mL plasma, add 500 μL 5 M NaOH and 5 mL ethyl acetate, extract. Centrifuge at 700 g for 10 min, evaporate 4 mL of the organic phase to dryness under nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 69:31 (Mobile phase was 690 mL MeCN, 310 mL water, and 9 mL glacial acetic acid, adjusted to pH 5.0 with 5 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 362 em 414

CHROMATOGRAM

Retention time: 1.8
Internal standard: prazosin (3.8)
Limit of detection: 250 pg/mL
Limit of quantitation: 500 pg/mL

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Jankowski,A.; Skorek-Jankowska,A.; Lamparczyk,H. Determination and pharmacokinetics of a furosemide-amiloride drug combination, *J.Chromatogr.B*, **1997**, *693*, 383–391.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL 10 mg/mL hydroflumethiazide in water + 1 mL 1 M pH 10 sodium carbonate-bicarbonate buffer + 5 mL ethyl acetate, vortex 1 min, centrifuge at 1250 g for 5 min. Remove the ethyl acetate layer and evaporate at 45° under nitrogen. Dissolve in 100 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 5 μm Spherisorb ODSII
Mobile phase: MeCN:MeOH:buffer 10:9:100 (Buffer was 15.54 g tetraethylammonium hydroxide and 2.9 g 89% orthophosphoric acid in 500 mL water, pH was 2.8.)
Flow rate: 1.2
Injection volume: 50
Detector: F ex 368 em 415

CHROMATOGRAM

Retention time: 3.67
Internal standard: hydroflumethiazide (7.94)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide (detection by UV)

KEY WORDS
plasma

REFERENCE
Van der Meer,M.J.; Brown,L.W. Simultaneous determination of amiloride and hydrochlorothiazide in plasma by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *423*, 351–357.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 8.5 M acetic acid, mix, add 250 μL 125 mM sodium dodecylsulfate, mix 5 s, add 100 μL 40 μg/mL naproxen in MeOH, add 7 mL ethyl acetate saturated with water, mix by rotation at 60 rpm for 30 min, centrifuge at 5200 g for 10 min. Remove organic phase and evaporate on a vortex evaporator at 35°. Dissolve in 250 μL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 5 μm Nucleosil 100 C18
Column: 100 × 3 5 μm Nucleosil 100 C18
Mobile phase: MeCN:125 mM sodium dodecylsulfate:10 mM pH 2.0 perchloric acid 234.6: 35:665
Flow rate: 0.6
Injection volume: 100
Detector: F ex 360 em 413

CHROMATOGRAM
Retention time: 8
Internal standard: naproxen (12)
Limit of detection: 0.03 ng/mL

OTHER SUBSTANCES
Simultaneous: furosemide

KEY WORDS
plasma

REFERENCE
Reeuwijk,H.J.; Tjaden,U.R.; van der Greef,J. Simultaneous determination of furosemide and amiloride in plasma using high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1992**, *575*, 269–274.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM
Retention time: 3.608

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 1.764

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1

Injection volume: 5
Detector: UV 254, F ex 286 em 418

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: bumetanide, furosemide, triamterene

KEY WORDS
column-switching

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM
Retention time: 3.1 (A), 4.0 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 5000 ng/mL

OTHER SUBSTANCES
Extracted: furosemide, metolazone, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, flumethiazide, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE
Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 4.5:10.5:85:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 2.1

Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES

Extracted: chlorthalidone, triamterene, furosemide, benzthiazide, bendroflumethiazide

REFERENCE

Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE

Matrix: urine

Sample preparation: Basify 2.5 mL urine to pH 12 with 100 mg potassium carbonate, extract twice with 5 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 15 μg/mL triamterene in MeOH:HCl 99:1, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2.1 30 μm HP ODS Hypersil

Column: 200 × 4.6 5 μm HP ODS Hypersil C18

Mobile phase: Gradient. A was 6.9 g $NaH_2PO_4.H_2O$ and 1.59 g propylamine hydrochloride in 1 L water, pH adjusted to 3.2 with concentrated phosphoric acid. B was MeCN. A:B at 85:15 for 2 min then to 20:80 over 18 min

Flow rate: 1

Injection volume: 10

Detector: UV 363

CHROMATOGRAM

Retention time: 3.7

Internal standard: triamterene (7.5)

Limit of detection: 120 ng/mL

KEY WORDS

pharmacokinetics

REFERENCE

Bi,H.; Cooper,S.F.; Côté,M.G. Determination and identification of amiloride in human urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1992**, *582*, 93–101.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50: 50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 360

CHROMATOGRAM

Retention time: 8.9

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 1.6

Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, bendroflumethiazide, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine
Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 5
Limit of detection: 20 ng/mL.

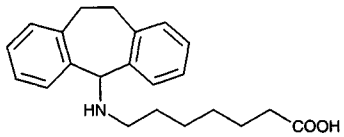
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Amineptine



Molecular formula: $C_{22}H_{27}NO_2$
Molecular weight: 337.46
CAS Registry No.: 57574-09-1, 30272-08-3 (HCl)
Merck Index: 429

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.032

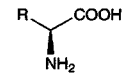
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Amino acids



Molecular formula: $C_2H_5NO_2$ (glycine), $C_3H_7NO_2$ (alanine), $C_3H_7NO_2S$ (cysteine), $C_3H_7NO_3$ (serine), $C_4H_7NO_4$ (aspartic acid), $C_4H_8N_2O_3$ (asparagine), $C_4H_9NO_3$ (threonine), $C_5H_{10}N_2O_3$ (glutamine), $C_5H_{11}NO_2$ (valine), $C_5H_{11}NO_2S$ (methionine), $C_5H_9NO_2$ (proline), $C_5H_9NO_3$ (hydroxyproline), $C_5H_9NO_4$ (glutamic acid), $C_6H_{13}NO_2$ (isoleucine), $C_6H_{13}NO_2$ (leucine), $C_6H_{14}N_2O_2$ (lysine), $C_6H_{14}N_4O_2$ (arginine), $C_6H_9N_3O_2$ (histidine), $C_9H_{11}NO_2$ (phenylalanine), $C_9H_{11}NO_3$ (tyrosine), $C_{11}H_{12}N_2O_2$ (tryptophan)

Molecular weight: 75.1 (glycine), 89.1 (alanine), 105.1 (serine), 115.1 (proline), 117.2 (valine), 119.1 (threonine), 121.2 (cysteine), 131.1 (hydroxyproline), 131.2 (isoleucine), 131.2 (leucine), 132.1 (asparagine), 133.1 (aspartic acid), 146.2 (lysine), 146.2 (glutamine), 147.1 (glutamic acid), 149.2 (methionine), 155.2 (histidine), 165.2 (phenylalanine), 174.2 (arginine), 181.2 (tyrosine), 204.2 (tryptophan)

CAS Registry No.: 51-35-4 (hydroxyproline), 52-89-1 (cysteine HCl), 52-90-4 (cysteine), 56-40-6 (glycine), 56-41-7 (alanine), 56-45-1 (serine), 56-84-8 (aspartic acid), 56-85-9 (glutamine), 56-86-0 (glutamic acid), 56-87-1 (lysine), 59-51-8 (DL-methionine), 60-18-4 (tyrosine), 61-90-5 (leucine), 63-68-3 (methionine), 63-91-2 (phenylalanine), 70-47-3 (asparagine), 71-00-1 (histidine), 72-18-4 (valine), 72-19-5 (threonine), 73-22-3 (tryptophan), 73-32-5 (isoleucine), 74-79-3 (arginine), 138-15-8 (glutamic acid HCl), 147-85-3 (proline), 150-30-1 (DL-phenylalanine), 302-72-7 (DL-alanine), 302-84-1 (DL-serine), 312-84-5 (D-serine), 338-69-2 (D-alanine), 348-67-4 (D-methionine), 513-29-1 (glycine sulfate), 585-21-7 (DL-glutamine), 609-36-9 (DL-proline), 617-65-2 (DL-glutamic acid), 673-06-3 (D-phenylalanine), 1119-34-2 (arginine HCl), 1783-96-6 (D-aspartic acid), 2058-58-4 (D-asparagine), 6893-26-1 (D-glutamic acid)

SAMPLE

Matrix: CSF

Sample preparation: 20 μL CSF + 0.4 μL reagent, mix, let stand for 10 min, inject an aliquot. (Prepare reagent by mixing 22 mg o-phthalaldehyde, 500 μL 1 M sodium sulfite, 500 μL EtOH, and 900 μL buffer. Prepare fresh each day, protect from light. The most satisfactory o-phthalaldehyde was obtained from Aldrich. Prepare buffer by adjusting the pH of 100 mM sodium tetraborate to 10.4 with 5 M NaOH.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Dynamax C18 (Rainin)

Mobile phase: MeOH:buffer 25:75, adjusted to pH 4.5 with 1 M phosphoric acid (Buffer was 100 mM NaH_2PO_4 containing 500 μM EDTA.)

Flow rate: 0.7

Injection volume: 20

Detector: E, Antec VT-03, glassy carbon working electrode +0.85 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 5 (serine), 6.5 (glycine), 7 (taurine), 8 (glutamic acid), 9 (arginine), 10 (alanine), 24.5 (gamma-aminobutyric acid)

Limit of detection: 5-10 fmole

OTHER SUBSTANCES

Noninterfering: aspartic acid, cysteine, leucine, methionine, phenylalanine, tryptophan, tyrosine

KEY WORDS

derivatization; rat

REFERENCE

Rowley,H.L.; Martin,K.F.; Marsden,C.A. Determination of in vivo amino acid neurotransmitters by high-performance liquid chromatography with *o*-phthalaldehyde-sulphite derivatisation, *J.Neurosci. Methods*, **1995**, *57*, 93–99.

SAMPLE

Matrix: amniotic fluid

Sample preparation: 200 μL Amniotic fluid + 800 μL MeOH, mix, centrifuge. 200 μL Supernatant + 80 μL pH 9.5 sodium borate + 60 μL reagent, mix, let stand for 3.5 min, add 25 μL 0.5 M HCl, mix, dilute 1:4 with 50 mM pH 7.0 sodium acetate buffer, inject a 20 μL aliquot. (Prepare reagent by dissolving 50 mg o-phthaldialdehyde in 4.5 mL MeOH, add 500 μL pH 9.5 sodium borate, add 50 μL 2-mercaptoethanol.)

HPLC VARIABLES

Guard column: 10-20 × 4 C18

Column: 300 × 3.9 5 μm NovaPak C18

Mobile phase: Gradient. MeOH:50 mM pH 7.0 sodium acetate buffer from 15:85 to 20:80 over 30 min, to 35:65 over 15 min, to 75:25 over 25 min, maintain at 75:25 for 5 min, return to initial conditions over 5 min.

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: F ex 330 em 450

CHROMATOGRAM

Retention time: 3 (glutathione), 5 (aspartic acid), 8.5 (glutatic acid), 14 (saccharopine), 17 (2-aminoadipic acid), 20 (asparagine), 23 (serine), 31 (glutamine), 35 (histidine), 37 (homoserine), 40 (glycine), 42.5 (threonine), 44 (citruline), 46 (arginine), 50 (taurine), 52 (alanine), 53.5 (4-aminobutyric acid), 54.5 (tyrosine), 57 (3-aminobutyric acid), 59 (2-aminobutyric acid), 61.5 (tryptophan), 62 (methionine), 63 (valine), 64 (phenylalanine), 66 (isoleucine), 67 (leucine), 68 (5-hydroxylysine), 70 (ornithine), 72 (lysine)

KEY WORDS

derivatization

REFERENCE

Klein,B.H.; Dudenhausen,J.W. Ion-exchange chromatography and ion-pair chromatography. Complementation of HPLC analysis of amino acids in body fluids by pre-column derivatization using ortho-phthaldialdehyde, *J.Liq.Chromatogr.*, **1995**, *18*, 4007–4028.

SAMPLE

Matrix: amniotic fluid, blood, CSF, urine

Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:

triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40. A:B 100:0 for 13.5 min, to 97:3 (step gradient), to 94:6 over 10.5 min (Waters curve 8 (slightly concave)), to 91:9 over 6 min (Waters curve 5 (slightly convex)), to 66:34 over 20 min, maintain at 66:34 for 12 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 0.5 min.
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.97 (phosphoserine), 3.08 (cysteic acid), 3.36 (aspartic acid), 3.80 (glutamic acid), 5.23 (α-aminoadipic acid), 6.83 (hydroxyproline), 7.21 (phosphoethanolamine), 8.70 (serine), 8.98 (galactosamine), 9.03 (aspartylglucosamine), 9.09 (asparagine), 9.75 (glycine), 9.91 (glucosamine), 10.46 (glutamine), 11.84 (β-alanine), 12.12 (homoserine), 12.12 (sarcosine), 12.56 (glycylglycine), 13.82 (taurine), 15.92 (histidine), 16.63 (gamma-aminobutyric acid), 17.02 (ammonia), 17.18 (citrulline), 18.23 (glycylhistidine), 18.83 (threonine), 19.88 (alanine), 21.26 (β-aminoisobutyric acid), 22.14 (carnosine), 22.25 (β-amino-n-butyric acid), 23.07 (arginine), 23.40 (methionine sulfone), 26.10 (proline), 26.71 (δ-amino-n-valeric acid), 27.48 (1-methylhistidine), 27.98 (anserine), 28.31 (homocitrulline), 28.36 (3-methylhistidine), 29.35 (4-aminobenzoic acid), 31.00 (ethanolamine), 31.89 (homoarginine), 33.59 (cysteine), 34.03 (gamma-amino-n-butyric acid), 34.03 (glutathionine (oxidized)), 34.14 (levodopa), 34.47 (Tris), 38.55 (4-aminophenylacetic acid), 40.37 (4-aminohippuric acid), 40.70 (glycyltyrosine), 42.29 (tyrosine), 45.38 (valine), 46.97 (methionine), 47.47 (3-hydroxyanthranilic acid), 48.74 (cystathionine), 48.90 (3-hydroxykynurenine), 50.28 (ethylamine), 50.83 (cystine), 51.05 (α-aminophenylacetic acid), 51.71 (glycylleucine), 51.77 (3-amino-3-phenylpropionic acid), 53.14 (isoleucine), 53.42 (alloisoleucine), 53.80 (leucine), 53.97 (cysteine-homocysteine (mixed disulfide)), 54.19 (ethionine), 54.79 (glycylphenylalanine), 56.12 (kynurenine), 56.12 (homocystine), 57.11 (phenylalanine), 57.99 (tryptophan), 58.43 (ornithine), 62.89 (lysine), 65.92 (serotonin)
Internal standard: norleucine (55.07)

OTHER SUBSTANCES

Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS

derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE

Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE

Matrix: ascitic fluid, blood

Sample preparation: 100 μL Plasma or ascitic fluid + 400 μL MeOH, shake gently, let stand at 4° for 10 min, centrifuge at 11600 g for 5 min, remove the supernatant, wash the precipitate with 100 μL MeOH:water 80:20, centrifuge at 11600 g for 5 min, remove the supernatant, wash the precipitate twice more. Combine the supernatants and evaporate them to 30-50 μL at 80°, make up to the original volume with water. Remove a 100 μL aliquot and add it to 100 μL 40 mM pH 9.5 lithium carbonate buffer, add 100 μL 20 mM dansyl chloride in MeCN, stir, let stand at room temperature for 1 h, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm New Guard RP-18

Column: 150 × 4.6 5 μm Supelcosil LC-18

Mobile phase: Gradient. A was MeOH. B was 0.6% acetic acid containing 0.008% triethylamine in water. A:B 30:70 for 20 min, to 40:60 over 5 min, to 50:50 over 25 min, maintain at 50:50 for 5 min, to 65:35 over 10 min, to 75:25 over 5 min, maintain at 75:25 over 5 min.

Flow rate: 1 for 25 min, to 1.5 for 25 min, to 2 over 5 min

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 3 (Cys), 3.5 (Cya), 4.5 (Tau), 12 (Asn), 15 (Gln), 17 (ammonia), 18.5 (Ser), 20 (Asp), 22 (Glu), 25 (Gly), 28 (Thr), 29 (β-alanine), 30 (Ala), 31.5 (GABA), 35.5 (α-ABA), 38 (Pro), 41 (Met), 43 (Val), 46 (Arg), 49 (Trp), 52 (Ile, Phe), 53.5 (Leu), 61 (Cis (bis-derivative)), 65 (Orn (bis-derivative)), 66 (Lys (bis-derivative)), 68 (His (bis-derivative)), 70 (Tyr (bis-derivative))

KEY WORDS

derivatization; mouse; plasma; protect from light

REFERENCE

Márquez,F.J.; Quesada,A.R.; Sánchez-Jiménez,F.; Núñez de Castro,I. Determination of 27 dansyl amino acid derivatives in biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *380*, 275–283.

SAMPLE

Matrix: beverages

Sample preparation: Filter (0.5 μm) wine, add 10 μL filtrate to 90 μL 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA, add 50 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, add 850 μL MeOH:acetic acid 99:1, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Tosoh)

Column: 250 × 4.6 5 μm Sumichiral OA-2500(R) or OA-2500(S) (1-naphthylglycyl-3,5-dinitrophenylamide silica) (Sumika)

Mobile phase: MeOH containing 4-5 mM citric acid

Flow rate: 0.8

Injection volume: 50

Detector: F ex 470 em 530

CHROMATOGRAM

Limit of detection: 2 nM

OTHER SUBSTANCES
Simultaneous: alanine, asparagine, aspartic acid, glutamic acid, glutamine, isoleucine, leucine, lysine

KEY WORDS
wine; chiral; derivatization

REFERENCE
Kato,M.; Fukushima,T.; Santa,T.; Homma,H.; Imai,K. Determination of D-amino acids, derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole, in wine samples by high-performance liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 193–194.

SAMPLE
Matrix: beverages, food
Sample preparation: Dilute orange juice or soy sauce with water, filter (0.2 μm) if necessary. 2.2 μL Solution + 5 μL 0.4 N pH 10.4 sodium borate buffer + 1 μL reagent, mix for 2 min, inject a 7 μL aliquot. (Reagent was 260 mM N-isobutyryl-L-cysteine and 170 mM o-phthaldialdehyde in 1 M pH 10.4 potassium borate buffer (Pierce fluoraldehyde diluent).

HPLC VARIABLES
Guard column: 20 × 4 5 μm Hypersil ODS
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. A was 23 mM sodium acetate adjusted to pH 6.00 with 10% acetic acid. B was MeOH:MeCN 60:5. A:B from 100:0 to 46.5:53.5 over 75 min, to 0:100 (step gradient), maintain at 0:100 for 10 min, re-equilibrate at initial conditions for 5 min.
Column temperature: 25
Flow rate: 1
Injection volume: 7
Detector: F ex 230 em 445

CHROMATOGRAM
Retention time: 20 (L-Asp), 21 (D-Asp), 27 (L-Glu), 28 (L-Asn), 28.5 (D-Glu), 29 (L-Ser), 30 (D-Asn), 32 (D-Ser), 33.5 (L-Gln), 35 (D-Gln), 36 (L-Thr), 37.5 (Gly), 38 (D-Thr), 39 (L-His), 40 (D-His), 43 (L-Ala), 44 (L-Arg), 45.5 (D-Arg), 46.5 (D-Ala), 48 (L-homo-Arg), 50 (L-Tyr), 52 (D-Tyr), 56.5 (L-Val), 57.5 (L-Met), 60 (L-Trp), 61 (D-Met), 62 (D-Val), 63 (L-Ile), 63.5 (L-Phe), 64.5 (D-Trp), 65.5 (D-Phe), 68 (L-Leu), 68.5 (D-Ile), 71 (D-Leu), 72.5 (L-Lys), 73.5 (D-Lys)
Limit of detection: 1 pmole

KEY WORDS
derivatization; orange juice; soy sauce; chiral

REFERENCE
Brückner,H.; Wittner,R.; Godel,H. Fully automated high-performance liquid chromatographic separation of DL-amino acids derivatized with *o*-phthaldialdehyde together with *N*-isobutyryl-cysteine. Application to food samples, *Chromatographia*, **1991**, *32*, 383–388.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL acetone, shake thoroughly, centrifuge at 5000 rpm for 10 min. Remove the supernatant and add it to 1 mL buffer and 5 μL phenylisothiocyanate, heat at 40° for 1 h, add 2 mL benzene (Caution! Benzene is a carcinogen!), shake, centrifuge at 3000 rpm for 3 min, discard the organic phase. Add 1 mL 1 M hydrogen chloride in acetic acid to the aqueous phase and flush the tube with nitrogen, heat at 80° for 50 min, cool, extract with two 10 mL aliquots of dichloromethane. Combine the extracts and evaporate them to dryness, reconstitute the residue with 5 mL dichloro-

methane and evaporate to dryness with a stream of nitrogen, reconstitute with 270 μL mobile phase, shake thoroughly, centrifuge at 3000 rpm for 3 min, inject a 200 μL aliquot of the supernatant. (Prepare the buffer by adding 2 mL 2 M acetic acid to 1.2 mL triethylamine and diluting with 96.8 mL acetone:water 50:50.)

HPLC VARIABLES

Column: 500 × 6 5 μm Lichrosorb Si 100
Mobile phase: n-Hexane:dichloromethane:saturated dichloromethane 25:55:20 (Prepare saturated dichloromethane by stirring 885 mL dichloromethane, 75 mL EtOH, and 40 mL water for 2 h, let stand for 2 h. The organic phase is saturated dichloromethane.)
Flow rate: 2.5
Injection volume: 200
Detector: UV 268

CHROMATOGRAM

Retention time: 4.8 (proline), 5.2 (leucine), 6.0 (valine), 6.2 (phenylalanine), 8.4 (alanine), 9.5 (tryptophan), 11.2 (glycine), 22.0 (tyrosine)

KEY WORDS

derivatization; plasma; normal phase; pharmacokinetics

REFERENCE

Trefz,F.K.; Byrd,D.J.; Blaskovics,M.E.; Kochen,W.; Lutz,P. Determination of deuterium-labeled phenylalanine and tyrosine in human plasma with high pressure liquid chromatography and mass spectrometry, *Clin.Chim.Acta*, **1976**, *73*, 431–438.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum or plasma + 450 μL cold MeOH + 10 μL 1 mM L-α-aminoadipate, vortex, centrifuge at 8700 g for 1 min. Remove the supernatant and evaporate it to dryness under reduced pressure at 45° over 1 h, reconstitute the residue in 25 μL water, 25 μL triethylamine, and 50 μL EtOH, evaporate to dryness under high vacuum over 30 min. Dissolve the residue in 70 μL EtOH, 10 μL water, 10 μL triethylamine, and 10 μL phenylisothiocyanate, let stand at room temperature for 20 min, evaporate to dryness under high vacuum over 1 h, reconstitute with 50 μL buffer, inject a 10 μL aliquot. (Buffer was MeCN:7 mM Na_2HO_4 5:95, adjusted to pH 7.4 with phosphoric acid.)

HPLC VARIABLES

Column: 250 × 4 Bio-sil ODS-5S (Bio-Rad)
Mobile phase: Gradient. A was 50 mM ammonium acetate adjusted to pH 6.8 with phosphoric acid. B was MeCN:100 mM pH 6.8 ammonium acetate 50:50. A:B from 100:0 to 92:8 over 40 min, to 40:60 over 40 min, wash with 0:100 for 15 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 6 (Asp), 7.5 (Glu), 12.5 (S-carboxmethyl-L-cysteine), 16.5 (OH-Pro), 20 (Ser), 21.5 (Gly), 23 (Asn), 27 (Glu), 35 (Thr), 37 (Ala), 40 (His), 45 (Pro), 49.5 (Arg), 59.5 (Tyr), 60 (Val), 62.5 (Met), 63 (Cys), 67 (Ile), 68 (Leu), 71 (Phe), 73 (Trp), 76 (Lys)
Internal standard: α-aminoadipate (14)
Limit of quantitation: 10 pmole

KEY WORDS

serum; plasma; derivatization

REFERENCE

Lavi,L.E.; Holcenberg,J.S.; Cole,D.E.; Jolivet,J. Sensitive analysis of asparagine and glutamine in physiological fluids and cells by precolumn derivatization with phenylisothiocyanate and reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *377*, 155–163.

SAMPLE

Matrix: blood

Sample preparation: Mix plasma with an equal volume of 5% trichloroacetic acid, centrifuge at 12000 rpm for 5 min. Evaporate the supernatant to dryness, reconstitute with 2-methoxyethanol:0.15 N pH 2.65 sodium citrate 7:93, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4 ISC-07/S1504 Li type (strongly acidic cation-exchange resin of styrene-divinylbenzene copolymer with 10% crosslinkage) (Shimadzu)

Mobile phase: Gradient. A was 2-methoxyethanol:0.15 N pH 2.65 sodium citrate 7:93. B was 0.3 N pH 10.0 sodium citrate. C was 200 mM NaOH. A:B:C from 100:0:0 to 95:5:0 over 50 min, to 85:15:0 over 20 min, to 75:25:0 over 10 min, to 55:45:0 (step gradient), maintain at 55:45:0 for 15 min, to 50:50:0 over 10 min, to 40:60:0 (step gradient), to 30:70:0 over 20 min, to 10:90:0 (step gradient), maintain at 10:90:0 for 15 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 5 min. (Parameters are approximate.)

Column temperature: 38° for 40 min, 52° for 65 min, 55° for 20 min, 58° for 10 min, 38° for 50 min

Flow rate: 0.4

Detector: F ex 348 em 450 following post-column reaction. The column effluent mixed with the reagent solution pumped at 0.2 mL/min and the mixture flowed through a 200 × 0.5 stainless steel or PTFE coil at 55°. The effluent from the coil mixed with the fluorescence solution pumped at 0.2 mL/min and flowed through a 2 m × 0.5 mm stainless steel or PTFE coil at 55° to the detector. (Prepare reagent solution by adding 400 μL NaOCl solution (chlorine concentration 10%) to 1 L buffer, discard after 2 weeks. Prepare fluorescence solution by adding 15 mL EtOH containing 1.6 g o-phthalaldehyde and 2.0 g N-acetyl-L-cysteine and 4 mL 10% Brij 35 in water to 980 mL buffer, discard after 1 month. Buffer contained 384 mM sodium carbonate, 216 mM boric acid, and 108 mM potassium sulfate, pH 10.0.)

CHROMATOGRAM

Retention time: 3.5 (phosphoserine), 5 (taurine), 5.5 (phosphoethanolamine), 16.5 (Asp), 22 (Thr), 24 (Ser), 27 (Asn), 28 (Glu), 33.5 (Sar), 40 (aminoadipic acid), 42 (Pro), 45 (Gly), 47 (Ala), 48 (citrulline), 49.5 (aminobutyric acid), 51.5 (Val), 62 (Met), 64 (Cys), 71 (Ile), 74 (Leu), 75 (cystathionine), 76.5 (Tyr), 80 (Phe), 87 (β-alanine), 88 (aminoisobutyric acid), 102.5 (His), 104 (3-methylhistidine), 105.5 (1-methylhistidine), 107.5 (carnosine), 109.5 (anserine), 125.5 (ornithine), 127 (ammonia), 131 (Lys), 145 (Arg)

Limit of quantitation: 10 pmole

KEY WORDS

derivatization; post-column reaction; plasma

REFERENCE

Fujiwara,M.; Ishida,Y.; Nimura,N.; Toyama,A.; Kinoshita,T. Postcolumn fluorometric detection system for liquid chromatographic analysis of amino and imino acids using o-phthalaldehyde/N-acetyl-L-cysteine reagent, *Anal.Biochem.*, **1987**, *166*, 72–78.

SAMPLE

Matrix: blood

Sample preparation: Dried blood. Add paper containing about 20 μL dried blood to 200 μL 500 μM norvaline in MeCN:triethylamine:water 50:25:15, rotate at 60-100 rpm for 30-60 min. Add 20 μL phenylisothiocyanate to the liquid, let stand at room temperature for 20-30 min, add 500 μL 100 mM pH 6.5 sodium acetate buffer, vortex for 30 s, add 2.5

mL dichloromethane, centrifuge, inject a 50 μL aliquot of the aqueous layer. Serum. 20 μL Serum + 200 μL 500 μM norvaline in MeCN:triethylamine:water 50:25:15, mix, centrifuge at 1500 g for 5 min. Add 20 μL phenylisothiocyanate to the supernatant, let stand at room temperature for 20-30 min, add 500 μL 100 mM pH 6.5 sodium acetate buffer, vortex for 30 s, add 2.5 mL dichloromethane, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C18
Mobile phase: Gradient. MeCN:100 mM pH 6.5 sodium acetate buffer 15:85 for 1 min, to 28:72 over 7 min, to 60:40 (step gradient), maintain at 60:40 for 5 min, return to initial conditions over 2 min.
Column temperature: 45
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3 (tyrosine), 8 (phenylalanine)
Internal standard: norvaline (4.5)
Limit of detection: <10 μM

OTHER SUBSTANCES
Noninterfering: other amino acids, bilirubin, lipids, carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone

KEY WORDS
derivatization; serum; dried blood

REFERENCE
Rudy,J.L.; Rutledge,J.C.; Lewis,S.L. Phenylalanine and tyrosine in serum and eluates from dried blood spots as determined by reversed-phase liquid chromatography, *Clin.Chem.*, **1987**, *33*, 1152–1154.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 300 μL MeCN, mix, centrifuge, lyophilize a 100 μL aliquot of the supernatant. Reconstitute with 50 μL 20 mM sodium bicarbonate solution, add 50 μL 5.1 mg/mL N,N-diethyl-2,4-dinitro-5-fluoroaniline (Fluka) in MeCN, heat at 100° for 15 min, evaporate to dryness under reduced pressure, reconstitute with 150 μL MeCN:buffer 50:50, inject a 15 μL aliquot. (Prepare buffer by adding 4 mL triethylamine and 3 mL acetic acid to water, make up to 1 L with water, adjusted to pH 4.3 with 5 M NaOH.)

HPLC VARIABLES
Guard column: ChromSpher C18 (Chrompack)
Column: 250 × 4.6 ChromSpher C18 (Chrompack)
Mobile phase: Gradient. MeCN:buffer 20:80 for 15 min, to 35:65 over 20 min, maintain at 35:65 for 15 min, to 45:55 over 20 min. (Prepare buffer by adding 4 mL triethylamine and 3 mL acetic acid to water, make up to 1 L with water, adjusted to pH 4.3 with 5 M NaOH.)
Flow rate: 2
Injection volume: 15
Detector: UV 360

CHROMATOGRAM
Retention time: 5 (Cya), 9 (hydroxyproline), 15.5 (Asn), 16 (Gln), 18 (Ser), 21.5 (Asp), 23.5 (Glu), 25 (Thr), 29 (Gly), 30 (Pro), 32 (Ala), 40 (Met), 42 (Val), 45.5 (Trp), 47 (Phe), 49 (Leu), 51 (Ile), 65 (Orn), 72 (Lys), 76 (Tyr)
Internal standard: 6-aminocaproic acid (60)

Limit of detection: 1 pmole

KEY WORDS
derivatization; plasma

REFERENCE
Fermo,I.; Rubino,F.M.; Bolzacchini,E.; Arcelloni,C.; Paroni,R.; Bonini,P.A. Pre-column derivatization of amino acids with N,N-diethyl-2,4-dinitro-5-fluoroaniline and reversed-phase liquid chromatographic separation, *J.Chromatogr.*, **1988**, *433*, 53–62.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 400 μL MeCN, mix, centrifuge. Remove a 40 μL aliquot of the supernatant and add it to 40 μL 4 nM dabsyl chloride in MeCN, heat at 70° for 10 min, add 70 μL MeOH:50 mM pH 7.0 phosphate buffer 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. A was MeCN:MeOH:100 mM pH 6.5 sodium acetate 16:28:56. B was MeCN:25 mM pH 6.5 sodium acetate 29:71. A:B 100:0 for 16 min, to 0:100 over 1 min, maintain at 0:100 for 18 min.
Flow rate: 1
Injection volume: 20
Detector: UV 436

KEY WORDS
derivatization; serum

REFERENCE
Jansen,E.H.J.M.; van den Berg,R.H.; Both-Miedema,R.; Doorn,L. Advantages and limitations of pre-column derivatization of amino acids with dabsyl chloride, *J.Chromatogr.*, **1991**, *553*, 123–133.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 5 μL 2.5 M homoserine, make up to 500 μL with 2 μL/mL 2-mercaptoethanol in MeCN, vortex, centrifuge for 4 min (Beckman microfuge). Remove a 40 μL aliquot of the supernatant and add it to 40 μL reagent and 20 μL 3.7% iodoacetic acid in 400 mM pH 9.5 sodium borate buffer, mix, let stand for 1 min, make up to 200 μL with 100 mM pH 4 potassium phosphate buffer, mix, inject a 20 μL aliquot. (Reagent was 50 mg o-phthaldialdehyde in 1 mL MeOH added to 11 mL 400 mM pH 9.5 sodium borate buffer, 50 μL 2-mercaptoethanol, and 10 mg nitrilotriacetic acid. Filter (0.2 μm), store in the dark at 4°, add 20 μL 2-mercaptoethanol each week to maintain the level of this reagent.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18
Column: 150 × 4.6 5 μm Dynamax Microsorb C18 (Rainin)
Mobile phase: Gradient. A was MeOH:100 mM pH 6.8 sodium acetate buffer 95:5. B was MeOH:100 mM pH 6.8 sodium acetate buffer 5:95. A:B from 15:85 to 30:70 over 15.5 min, to 55:45 over 9 min, to 60:40 over 2 min, to 100:0 over 8 min, maintain at 100:0 for 3 min, to 0:100 over 4 min, maintain at 0:100 for 3 min, to 15:85 over 1 min, stay at 15:85 for 2 min.
Column temperature: 35
Flow rate: 1.5 for 37.5 min, 1 for 10 min
Injection volume: 20
Detector: F ex 338 em 425

CHROMATOGRAM

Retention time: 6.37 (aspartic acid), 7.72 (glutamic acid), 10.81 (asparagine), 12.25 (serine), 14.20 (glutamine), 15.11 (histidine), 17.75 (glycine), 18.56 (threonine), 20.83 (arginine), 22.60 (taurine), 24.58 (alanine), 25.88 (tyrosine), 31.25 (tryptophan), 31.41 (methionine), 31.90 (valine), 32.75 (phenylalanine), 34.31 (isoleucine), 34.88 (leucine), 36.92 (lysine)
Internal standard: homoserine (16.31)
Limit of quantitation: 31 μM

KEY WORDS

plasma; derivatization

REFERENCE

Uhe,A.M.; Collier,G.R.; McLennan,E.A.; Tucker,D.J.; O'Dea,K. Quantitation of tryptophan and other plasma amino acids by automated pre-column o-phthaldialdehyde derivatization high-performance liquid chromatography: improved sample preparation, *J.Chromatogr.*, **1991**, *564*, 81–91.

SAMPLE

Matrix: blood
Sample preparation: Filter (Amicon Centrifree) while centrifuging at 1500 g for 15 min, mix 2.5 μL ultrafiltrate with 2.5 μL reagent, add 1 μL 1 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 2.5 min, inject the whole amount. (Prepare reagent by dissolving 3 mg o-phthalaldehyde in 50 μL MeOH, add 450 μL 0.5 M pH 10.2 sodium borate buffer, add 5 μL 3-mercaptopropionic acid. Derivatization was performed automatically and took 5 min. o-Phthalaldehyde derivatized primary amino acids and 9-fluorenylmethyl chloroformate derivatized secondary amino acids (proline and hydroxyproline).)

HPLC VARIABLES

Guard column: 20 × 2.1 5 μm Hypersil ODS
Column: two 100 × 2.1 5 μm Hypersil ODS columns in series
Mobile phase: Gradient. 15 mM pH 6.8 Sodium acetate:MeOH:10 mM pH 6.8 sodium acetate from 0:0:100 to 100:0:0 over 0.05 min, to 60:40:0 over 15 min, to 57.5:42.5:0 over 3.5 min, to 45:55:0 over 3.5 min, to 0:0:100 over 3 min, maintain at 0:0:100 for 5 min.
Column temperature: 40
Flow rate: 0.3
Injection volume: 6
Detector: F ex 230 em 450, after 20 min F ex 260 em 315

CHROMATOGRAM

Retention time: 1.8 (O-phospho-L-serine), 2 (aspartic acid), 2.5 (glutamic acid), 5 (glutathione (reduced)), 6 (asparagine), 6.2 (serine), 7.5 (glutamine), 8 (glycine), 8.5 (threonine), 8.8 (histidine), 9.2 (cystine), 9.5 (citrulline), 10.2 (taurine), 10.5 (alanine), 11.5 (arginine), 12.3 (tyrosine), 13.2 (α-amino-N-butyric acid), 15.3 (methionine), 15.5 (valine), 16 (norvaline), 16.2 (tryptophan), 16.5 (phenylalanine), 17.8 (isoleucine), 18.2 (ornithine), 18.5 (leucine), 19.5 (lysine), 20.5 (hydroxyproline), 22.3 (sarcosine), 24.5 (proline)
Limit of detection: 5 pmole

KEY WORDS

derivatization; plasma; ultrafiltrate

REFERENCE

Worthen,H.G.; Liu,H. Automatic pre-column derivatization and reversed-phase high performance liquid chromatography of primary and secondary amino acids in plasma with photo-diode array and fluorescence detection, *J.Liq.Chromatogr.*, **1992**, *15*, 3323–3341.

SAMPLE

Matrix: blood

Sample preparation: 90 μL Serum + 10 μL 2.5 mM cyclohexylalanine + 200 μL ethyl acetate + 2 μL benzoyl chloride + 6 μL triethylamine, vortex at 2500 vibrations/min for 2 min. Remove 150 μL of the ethyl acetate phase and evaporate it to dryness, dissolve the residue in 100 μL MeCN:water 70:30, inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm Kromasil 100 C18
Column: 250 × 4 5 μm Kromasil 100 C18
Mobile phase: Gradient. MeCN:water from 70:30 to 95:5 over 30 min.
Flow rate: 1
Injection volume: 50
Detector: UV 274 or MS, electrospray, Finnigan MAT, TSQ 700, flow rate 1 μL/min, 2.8 kV, drying gas 140

CHROMATOGRAM

Retention time: 6.4 (lysine), 7.1 (glycine), 7.5 (alanine), 10.4 (glutamate), 8.5 (tryptophan), 8.5 (cystine), 9.4 (methionine), 11.8 (phenylalanine), 12.1 (valine), 13.3 (cysteine), 13.9 (leucine), 15.1 (isoleucine), 15.2 (naphthylalanine), 15.5 (tyrosine)
Internal standard: cyclohexylalanine (21.0)
Limit of quantitation: 10 pmol

KEY WORDS

serum; derivatization; fetal bovine serum

REFERENCE

Oehlke,J.; Brudel,M.; Blasig,I.E. Benzoylation of sugars, polyols and amino acids in biological fluids for high-performance liquid chromatographic analysis, *J.Chromatogr.B*, **1994**, *655*, 105–111.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5% perchloric acid, centrifuge at 3000 rpm for 10 min. Remove the supernatant and neutralize it with 3 M potassium carbonate, centrifuge at 3000 rpm for 5 min, adjust the volume to 2 mL. (Alternatively, filter (Amicon CF-50) 4 mL plasma while centrifuging at 2500 rpm for 10 min.) 10 μL Perchloric acid extract or ultrafiltrate + 10 μL 100 mM pH 9.0 sodium bicarbonate + 40 μL freshly prepared 4 mM 4-dimethylaminoazobenzene-4'-sulfonyl chloride in MeCN, heat at 70° for 10 min, cool, make up to 500 μL with EtOH:water 70:30, centrifuge at 14000 rpm for 3 min, inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 T
Column: 150 × 4.6 3 μm Supelcosil LC-18 T
Mobile phase: Gradient. A was 25 mM pH 6.8 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 80:20 for 1 min, to 77:23 over 4 min, maintain at 77:23 for 7 min, to 73:27 over 11 min, to 70:30 over 7 min, to 40:60 over 9 min, to 30:70 over 1 min, maintain at 30:70 for 5 min, return to initial conditions over 1 min, re-equilibrate for 6 min.
Flow rate: 1.5
Injection volume: 5
Detector: UV 436

CHROMATOGRAM

Retention time: 17-42

OTHER SUBSTANCES

Extracted: taurine

KEY WORDS

plasma; ultrafiltrate; derivatization

REFERENCE
Stocchi,V.; Palma,F.; Piccoli,G.; Biagarelli,B.; Cucchiarini,L.; Magnani,M. HPLC analysis of taurine in human plasma sample using the DABS-Cl reagent with sensitivity at picomole level, *J.Liq.Chromatogr.*, **1994**, *17*, 347–357.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 200 mg/mL 5-sulfosalicylic acid in EtOH to a 1 mL tube, evaporate EtOH at 50° overnight, add 200-500 μL plasma, vortex, freeze in liquid nitrogen, store at -70°, thaw, centrifuge at 4° at 3000 g. 5 μL Supernatant + 20 μL water + 5 μL 1 mM norvaline in water + 90 μL reagent, mix thoroughly, incubate at room temperature for 3 min, add 50 μL neutralizing buffer, inject a 3 μL aliquot. (Prepare reagent stock solution by dissolving 25 mg o-phthalaldehyde in 500 μL MeOH, add 4.5 mL 100 mM pH 10.0 borate buffer, add 25 μL 3-mercaptopropionic acid. At the start of each day prepare reagent by diluting 1 part of stock solution with 20 parts 100 mM pH 10.0 borate buffer. Neutralizing buffer was 400 mM KH_2PO_4 containing 10 mL/L triethylamine.)

HPLC VARIABLES
Guard column: 10 × 2 Chrompack reverse phase
Column: 100 × 4.6 3 μm Microsphere C18 (Chrompack)
Mobile phase: Gradient. A was buffer:water:THF 50:50:0.2. B was MeOH:MeCN:buffer 35:15:50. A:B from 98:2 to 75:25 over 3.5 min, to 56:44 over 1.7 min, to 48:52 over 1.7 min, to 0:100 over 3.1 min, reset to initial conditions over 1 min.
Flow rate: 1.5
Injection volume: 3
Detector: F ex 230 em 389 (cut-off filter)

CHROMATOGRAM
Retention time: 2.3 (Asp), 4.1 (Glu), 4.8 (Asn), 5.1 (Ser), 5.8 (Gln), 6.2 (Gly), 6.4 (Thr), 6.6 (His), 6.8 (Cit), 7.0 (1-methylhistidine), 7.2 (3-methylhistidine), 7.4 (Ala), 7.6 (Tau), 7.7 (Arg), 8.5 (Tyr), 8.7 (α-aminobutyric acid), 9.8 (Val), 10.0 (Met), 10.5 (Trp), 10.7 (Phe), 11.0 (Ile), 11.2 (Orn), 11.4 (Leu), 11.6 (Lys)
Internal standard: norvaline (10.3)
Limit of quantitation: 5000 nM

KEY WORDS
plasma

REFERENCE
Teerlink,T.; Van Leeuwen,P.A.M.; Houdijk,A. Plasma amino acids determined by liquid chromatography within 17 minutes, *Clin.Chem.*, **1994**, *40*, 245–249.

SAMPLE
Matrix: blood
Sample preparation: 5 μL Plasma + 200 μL 20 mM pH 8.0 phosphate buffer, filter (Advantec Q0100, molecular mass cutoff), inject a 20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 4 5 μm Capcell C18 SG120
Mobile phase: 20 mM pH 7.5 phosphate buffer
Column temperature: 50
Flow rate: 0.4
Injection volume: 20
Detector: chemiluminescence following post-column reaction. The column effluent mixed with 5 mM niacinamide adenine dinucleotide in 20 mM pH 7.0 phosphate buffer pumped at 0.15 mL/min and 5 mM luminol in carbonate buffer pumped at 0.15 mL/min and this mixture flowed through an immobilized-enzyme reactor at 50°. The effluent from the re-

actor mixed with 20 mM potassium hexacyanoferrate(III) in water pumped at 0.4 mL/min and this mixture flowed through a 90 cm × 0.5 mm ID tube to the detector. (The carbonate buffer was 400 mM sodium carbonate containing 400 mM sodium bicarbonate, pH 10.5. Prepare the immobilized enzyme reactor as follows. Wash 1 g 13 μm poly(vinyl alcohol) beads (GS-520, Showa Denko, Tokyo) with 50 mL dry acetone, suspend in 20 mL dry acetone:pyridine 50:50 with vigorous stirring, add 1 mL 2,2,2-trifluoroethanesulfonyl chloride dropwise over 2 min, stir for 10 min, wash beads with 10 mL acetone, wash beads with 20 mL 1 mM HCl, slurry pack in a 50 × 4 column, circulate enzyme solution through the column at 0.2 mL/min for 4 h, monitor the immobilization process at 380 nm. The enzyme solution consisted of 5 mg (325 U) LeuDH (from Bacillus stearothermophilus, Unitika, Osaka) and 5 mg (280 U) NAOD (from Bacillus megaterium, Unitika, Osaka) in 10 mL 100 mM pH 7.0 phosphate buffer. When not in use store the reactor in 5 mM nicotinamide adenine dinucleotide in 20 mM pH 7.0 phosphate buffer at 5°.)

CHROMATOGRAM

Retention time: 8 (valine), 10.8 (isoleucine), 11.6 (leucine)
Limit of detection: 100 nM

KEY WORDS

post-column reaction; plasma; ultrafiltrate; immobilized enzyme reactor

REFERENCE

Kiba,N.; Oyama,Y.; Kato,A.; Furusawa,M. Postcolumn co-immobilized leucine dehydrogenase-NADH oxidase reactor for the determination of branched-chain amino acids by high-performance liquid chromatography with chemiluminescence detection, *J.Chromatogr.A*, **1996**, *724*, 354–357.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL MeCN, vortex, let stand for 10 min, centrifuge at 12000 rpm for 15 min. Remove a 10 μL aliquot of the supernatant and add it to 70 μL 200 mM pH 8.8 borate buffer, vortex, add 20 μL 3 mg/mL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters) in MeCN, vortex immediately, heat at 50° for 10 min, dilute (if necessary), inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC AP303 300 Å ODS (YMC)
Mobile phase: Gradient. A was MeCN:water:buffer 2:96:2. B was MeCN:water:buffer 60:38:2. A:B 100:0 for 2 min, to 70:30 over 40 min, maintain at 70:30 for 10 min. (Prepare buffer by adjusting the pH of 1 M NaH_2PO_4 to 3.0 with phosphoric acid.)
Flow rate: 1.5
Detector: E, Bioanalytical Systems LC-4B, Model MF-1000 glassy carbon working electrode +1.1 V, stainless steel counter electrode, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.2 (ammonia), 16.8 (His), 18.5 (Ser), 19.2 (Arg), 19.5 (Gly), 20.8 (Asp), 22 (Glu), 23.2 (Thr), 24.8 (Ala), 27.2 (Pro), 33 (Cys), 34.8 (Lys), 36.8 (Met), 36.8 (Tyr), 44.8 (Ile), 45.6 (Leu), 47.2 (Phe), 50.4 (Trp)
Limit of detection: 250 nM
Limit of quantitation: 500 nM

KEY WORDS

plasma; derivatization; cow; human

REFERENCE

Li,G.D.; Krull,I.S.; Cohen,S.A. Electrochemical activity of 6-aminoquinolyl urea derivatives of amino acids and peptides. Application to high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *724*, 147–157.

SAMPLE

Matrix: blood, CSF

Sample preparation: Plasma. For each volume of plasma add 4 volumes of MeOH, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde/β-mercaptoethanol derivatizing reagent (Fluoraldehyde, Pierce) (use fresh reagent), allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot. CSF. Add an equal volume of MeOH to the CSF, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde, allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 Spherisorb 5 ODS

Column: 50 × 4.6 Spherisorb 5 ODS

Mobile phase: Gradient. A was THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid. B was MeOH:THF 95:5. A:B from 10:90 to 0:100 over 13 min (sic), maintain at 0:100 for 4 min, return to initial conditions over 1 min.

Column temperature: 43

Flow rate: 1.5

Injection volume: 20

Detector: F (wavelengths not specified)

CHROMATOGRAM

Retention time: 0.5 (Asp), 0.7 (Glu), 1.2 (Tau), 1.5 (Ser), 1.8 (Gln), 2.2 (His), 2.3 (Gly), 3.0 (Thr), 3.1 (Asn), 3.2 (Ala), 3.3 (Arg), 3.8 (Tyr), 5.2 (Met), 5.4 (Val), 5.8 (Trp), 6.0 (Phe), 5.5 (Ile), 5.7 (Leu), 8.3 (Orn), 8.5 (Lys)

Limit of detection: 10 nM

KEY WORDS

plasma; Thr; Gly; His co-elute; derivatization

REFERENCE

Begley,D.J.; Reichel,A.; Ermisch,A. Simple high-performance liquid chromatographic analysis of free primary amino acid concentrations in rat plasma and cisternal cerebrospinal fluid, *J.Chromatogr.B*, **1994**, *657*, 185–191.

SAMPLE

Matrix: blood, CSF, plants, tissue

Sample preparation: Seeds, serum, CSF. Homogenize seeds in 100 mL ice-cold water, centrifuge at 4° at 25000 g for 30 min. Mix seed homogenate, serum, or CSF with an equal volume of 10% trichloroacetic acid, centrifuge at 4° at 14000 g for 30 min, filter (0.2 μm) the supernatant. Dilute the supernatant with an equal volume of water. Remove a 20 (seed) or 30 (serum, CSF) μL aliquot and add it to 1 mL 25 mM pH 9.6 borate buffer, add 1 mL acetone, add 15 μL 100 μg/mL D-aminovaleric acid, add 100 μL 10 mM 9-fluorenylmethyl chloroformate in acetone (freshly prepared), vortex for 30 s, let stand at room temperature for 10 min, add 2 mL hexane:ethyl acetate 50:50, vortex for 30 s, inject a 20 μL aliquot of the aqueous layer. Tissue. Sonicate rat sensorimotor cortex with 2 mL ice-cold PBS with two 10 s bursts at 0°. Remove a 200 μL aliquot of the homogenate and add it to 100 μL 10% trichloroacetic acid, mix, centrifuge at 4° at 25000 g for 30 min. Remove a 30 μL aliquot and add it to 1 mL 25 mM pH 9.6 borate buffer, add 1 mL acetone, add 15 μL 100 μg/mL D-aminovaleric acid, add 100 μL 10 mM 9-fluorenylmethyl chloroformate in acetone (freshly prepared), vortex for 30 s, let stand at room temperature for 10 min, add 2 mL hexane:ethyl acetate 50:50, vortex for 30 s, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: Gradient. MeCN:50 mM pH 3.65 sodium acetate buffer from 31:69 to 48:52 over 19 min, to 70:30 over 3 min (non-linear gradient), return to initial conditions over 8 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 315

CHROMATOGRAM

Retention time: 2.5 (arginine), 3.5 (aspartate, serine), 4.5 (glutamate), 5 (threonine), 6.2 (glycine), 8 (alanine), 8.5 (tyrosine), 9.5 (gamma-aminobutyric acid), 10 (proline), 11.5 (methionine), 12.5 (valine), 14.5 (phenylalanine), 15.4 (isoleucine), 15.7 (leucine), 17.5 (β-N-methylamino-L-alanine), 18 (histidine), 18.5 (lysine, cystine)
Internal standard: D-aminovaleric acid (11)
Limit of detection: 0.6 pmole

KEY WORDS

derivatization; rat; monkey; brain; serum; seeds

REFERENCE

Kisby,G.E.; Roy,D.N.; Spencer,P.S. Determination of β-*N*-methylamino-L-alanine (BMAA) in plant (*Cycas circinalis* L.) and animal tissue by precolumn derivatization with 9-fluorenylmethyl chloroformate (FMOC) and reversed-phase high-performance liquid chromatography, *J.Neurosci.Methods*, **1988**, *26*, 45–54.

SAMPLE

Matrix: blood, CSF, tissue
Sample preparation: Blood. Mix plasma or whole blood with an equal volume of 0.5% sodium dodecyl sulfate, let stand for 15 min, add 2 volumes of 6% perchloric acid, add norleucine, centrifuge. Remove a 100 μL aliquot of the supernatant and add it to 25 μL buffer, add 125 μL freshly-prepared 2.5 μL/mL 1-naphthyl isocyanate in acetone, mix, let stand for 45 s, add 1 mL cyclohexane, vortex vigorously, discard the cyclohexane layer, repeat the wash twice more, centrifuge the aqueous layer, inject a 20 μL aliquot of the supernatant. CSF. Mix 1 volume of 60% perchloric acid with 20 volumes of CSF, centrifuge. Remove a 100 μL aliquot of the supernatant and add it to 25 μL buffer, add 125 μL freshly-prepared 2.5 μL/mL 1-naphthyl isocyanate in acetone, mix, let stand for 45 s, add 1 mL cyclohexane, vortex vigorously, discard the cyclohexane layer, repeat the wash twice more, centrifuge the aqueous layer, inject a 20 μL aliquot of the supernatant. Tissue. Homogenize brain tissue with 9 volumes 3% perchloric acid, centrifuge at 9000 g for 3 min. Remove the supernatant and neutralize it with NaOH, add 3 volumes of water, add norleucine. Remove a 100 μL aliquot and add it to 25 μL buffer, add 125 μL freshly-prepared 2.5 μL/mL 1-naphthyl isocyanate in acetone, mix, let stand for 45 s, add 1 mL cyclohexane, vortex vigorously, discard the cyclohexane layer, repeat the wash twice more, centrifuge the aqueous layer, inject a 5 μL aliquot of the supernatant. (Prepare buffer by adjusting the pH of 1 M boric acid to 6.25 with NaOH. Dry acetone over anhydrous sodium sulfate.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Absorbosphere HS C18
Mobile phase: Gradient. A was MeCN:MeOH:buffer:water 2.5:12.5:4.5:80.5. B was MeCN:buffer:water 45:4:51. C was MeCN:water 70:30. A:B:C 100:0:0 for 45 min, to 0:100:0 over 150 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 10 min, re-equilibrate at initial conditions for 30 min. (Buffer was 100 mM NaH_2PO_4 containing 100 mM sodium acetate, adjusted to pH 5.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-20
Detector: F ex 228 em 320 (cut-off filter), UV 225

CHROMATOGRAM

Retention time: 27 (Asp), 34 (Glu), 44 (Asn), 50 (Ser), 57 (Gly), 60 (Gln), 65 (Tau), 69 (His), 78 (Thr), 81 (Pro), 83 (Ala), 88 (Arg), 90 (GABA), 95 (ammonia), 105 (glutathione), 109 (Tyr), 116 (Val), 118 (Met), 130 (Ile), 131 (Leu (some interference from matrix)), 136 (Phe), 139 (Trp), 140 (Cys), 154 (Orn), 158 (Lys)

Internal standard: norleucine (133)

KEY WORDS

derivatization; plasma; whole blood; brain; human; mouse

REFERENCE

Neidle,A.; Banay-Schwartz,M.; Sacks,S.; Dunlop,D.S. Amino acid analysis using 1-naphthylisocyanate as a precolumn high performance liquid chromatography derivatization reagent, *Anal.Biochem.*, **1989**, *180*, 291–297.

SAMPLE

Matrix: blood, food, peptides, plants, tissue

Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES

Guard column: present but not specified

Column: 150 × 3.9 4 μm Novapak C18

Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.

Column temperature: 50

Flow rate: 1

Injection volume: 10

Detector: UV 436

CHROMATOGRAM

Retention time: 13.95 (O-phosphoserine), 14.94 (aspartic acid), 15.15 (O-phosphothreonine), 15.91 (glutamic acid), 16.39 (carboxymethylcysteine), 16.89 (S-sulfocysteine), 17.10 (β-aminoadipic acid), 22.51 (hydroxyproline), 23.13 (asparagine), 24.72 (glutamine), 25.35 (citrulline), 26.17 (serine), 27.43 (phosphoethanolamine), 27.69, 28.15 (methionine sulfoxide (diastereomers)), 28.45 (threonine), 28.94 (glycine), 28.94 (1- and 3-methylhistidine), 29.38 (arginine), 30.65 (alanine), 30.92 (β-alanine), 31.52 (anserine), 32.31 (taurine), 32.58 (sarcosine), 32.75 (α-aminobutyric acid), 33.27 (gamma-aminobutyric acid), 33.99 (proline), 34.40 (β-aminoisobutyric acid), 35.55 (valine), 37.60 (methionine), 39.35 (isoleucine), 40.03 (leucine), 40.51 (tryptophan), 41.57 (phenylalanine), 42.05 (ammonia), 43.62 (lanthionine), 44.00 (agmatine), 44.76 (2-aminoethanol), 44.76 (cystathionine), 45.65 (cyste-

ine), 46.83 (homocysteine), 48.02 (1-amino-2-propanol), 50.04 (hydroxylysine), 51.02 (ornithine), 51.58 (lysine), 52.10 (histidine), 52.38 (carnosine), 52.59 (ethylamine), 54.30 (tyrosine), 57.22 (pyrrolidine), 57.22 (tryptamine), 57.79 (isobutylamine), 58.34 (3,4-dihydroxyphenylalanine), 59.20 (phenylethylamine), 59.94 (methylbutylamine), 62.16 (putrescine), 63.29 (cadaverine), 63.87 (histamine), 63.87 (cystamine), 65.00 (serotonin), 67.31 (tyramine), 67.98 (spermidine), 68.49 (norepinephrine), 69.05 (dopamine), 70.61 (epinephrine), 71.94 (spermine)
Internal standard: norleucine (40.90), norvaline (35.06)
Limit of detection: 0.12-0.52 pmole
Limit of quantitation: 0.4-1.5 pmole

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, juice, urine
Sample preparation: Serum, urine. 400 μL Serum or urine + 50 μL 30% 5-sulfosalicylic acid + 50 μL 0.52 mM L-homo-Arg, centrifuge at 6000 g. Remove a 2 μL aliquot and add it to 5 μL 0.4 N pH 10.4 sodium borate buffer and 1 μL reagent, mix for 2 min, inject a 7 μL aliquot. Juice. Centrifuge filtered (paper) apple juice at 1650 g, remove a 1 mL aliquot and add it to 31.3 μL 1.6 mM L-homo-Arg in 100 mM HCl, adjust pH to 2.0 with 2 M HCl, add to a 50 × 10 column of Dowex 50W-X8 cation-exchanger, wash with water, elute with 30 mL 4 M aqueous ammonia, evaporate eluate to dryness, dissolve the residue in 2 mL 100 mM HCl. Remove a 2 μL aliquot and add it to 5 μL 0.4 N pH 10.4 sodium borate buffer and 1 μL reagent, mix for 2 min, inject a 7 μL aliquot. (Reagent was 260 mM N-isobutyryl--L-cysteine and 170 mM o-phthaldialdehyde in 1 M pH 10.4 potassium borate buffer (fluoraldehyde, Pierce) (Chromatographia 1991, 32, 383).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil ODS
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. A was 3.13 g sodium acetate trihydrate in 990 mL water adjusted to pH 5.95 with 10% acetic acid, make up to 1 L. B was MeCN:MeOH 50:600. A:B from 100:0 to 46.5:53.5 over 75 min, re-equilibrate at 100:0 for 10 min.
Column temperature: 25
Flow rate: 1
Injection volume: 7
Detector: F ex 230 em 445 (280 nm cut-off filter)

CHROMATOGRAM
Retention time: 18.89 (L-Asp), 20.09 (D-Asp), 25.87 (L-Glu), 27.54 (D-Glu), 26.95 (L-Asn), 29.30 (D-Asn), 28.46 (L-Ser), 30.61 (D-Ser), 32.41 (L-Gln), 34.01 (D-Gln), 34.59 (L-Thr), 36.78 (D-Thr), 36.21 (Gly), 37.76 (L-His), 39.15 (D-His), 41.95 (L-Ala), 45.27 (D-Ala), 43.29 (L-Arg), 44.66 (D-Arg), 48.29 (L-Tyr), 50.87 (D-Tyr), 55.36 (L-Val), 60.71 (D-Val), 56.30 (L-Met), 59.65 (D-Met), 58.53 (L-Trp), 63.08 (D-Trp), 61.80 (L-Phe), 64.39 (D-Phe), 62.33 (L-Ile), 67.56 (D-Ile), 66.45 (L-Leu), 69.87 (D-Leu), 71.41 (L-Lys), 72.69 (D-Lys)
Internal standard: L-hono-Arg (47.5)

KEY WORDS
serum; human; dog; apple; chiral; derivatization

REFERENCE

Brückner,H.; Haasmann,S.; Langer,M.; Westhauser,T.; Wittner,R.; Godel,H. Liquid chromatographic determination of D- and L-amino acids by derivatization with o-phthaldialdehyde and chiral thiols Applications with reference to biosciences, *J.Chromatogr.A*, **1994**, *666*, 259–273.

SAMPLE

Matrix: blood, protein

Sample preparation: Plasma. Mix plasma vigorously with 3 volumes MeCN, centrifuge at 12000 g for 3 min, evaporate an aliquot of the supernatant to dryness under reduced pressure, reconstitute with buffer. Remove a 5 μL aliquot and add it to 5 μL 4.16 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 1.5 min, add 5 μL reagent, mix, let stand for 3.5 min, add 5 μL MeCN:water:acetic acid 80:12:8, mix, inject an aliquot. Protein. Hydrolyse protein with 500 μL 6 M HCl at 110° for 24 h, evaporate to dryness under reduced pressure, add 10 μL triethylamine:EtOH:water 40:40:20, evaporate to dryness, reconstitute with 5 μL buffer, add 5 μL 4.16 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 1.5 min, add 5 μL reagent, mix, let stand for 3.5 min, add 5 μL MeCN:water:acetic acid 80:12:8, mix, inject an aliquot. (Buffer was 200 mM boric acid adjusted to pH 8.5 with 5 M NaOH. Prepare reagent by mixing 170 μL 850 mM NaOH, 75 μL 500 mM hydroxylamine hydrochloride, and 5 μL 2-(methylthio)ethanol.)

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard ODS

Column: 150 × 4.6 3 μm Spherisorb ODS-2

Mobile phase: Gradient. A was MeOH:20 mM pH 6.5 $(NH_4)H_2PO_4$ 15:85. B was MeCN: water 90:10. A:B 82:18 for 2 min, to 77:23 over 1 min, maintain at 77:23 for 7 min, to 64: 36 over 10 min, to 52:48 over 1 min, maintain at 52:48 for 5 min, to 45:55 over 2 min, to 1:99 over 1 min (plasma). A:B 82:18 for 2 min, to 77:23 over 1 min, maintain at 77:23 for 3 min, to 60:40 over 10 min, to 55:45 over 1 min, maintain at 55:45 for 3 min, to 45:55 over 2 min, to 1:99 over 1 min (protein hydrolysate).

Column temperature: 35

Flow rate: 1

Detector: F ex 263 em 313, UV 263

CHROMATOGRAM

Retention time: 3 (phosphoserine), 3.5 (aspartic acid), 3.7 (glutamic acid), 4 (α-aminoadipic acid), 4.2 (S-carboxymethylcysteine), 6.7 (hydroxyproline), 7.5 (asparagine), 8.5 (glutamine), 8.7 (citrulline), 9 (serine), 9.5 (histidine), 10 (glycine), 10.6 (threonine), 11.2 (β-alanine), 11.7 (alanine), 12.3 (taurine), 13 (proline), 14.5 (tyrosine), 15 (α-aminobutyric acid), 15.8 (arginine), 17 (homoarginine), 17.7 (valine), 18.3 (methionine), 20.5 (isoleucine), 21 (leucine), 21.5 (norleucine), 22 (phenylalanine), 23 (cystathionine), 26.3 (ornithine), 27 (lysine, using plasma gradient.)

Limit of detection: 50 fmole

KEY WORDS

derivatization; plasma

REFERENCE

Haynes,P.A.; Sheumack,D.; Greig,L.G.; Kibby,J.; Redmond,J.W. Applications of automated amino acid analysis using 9-fluorenylmethyl chloroformate, *J.Chromatogr.*, **1991**, *588*, 107–114.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Mix 9 volumes of plasma with 1 volume of 35% 5-sulfosalicylic acid, centrifuge at 2000 g for 10 min. Neutralize the supernatant with 10 M KOH, dilute with 2 volumes of water. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. Tissue. Homogenize tissue with four volumes 5% 5-sulfo-

salicylic acid, centrifuge at 5000 g for 10 min, neutralize the supernatant with 10 M KOH. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. (Prepare reagent each day by dissolving 35 mg o-phthalaldehyde in 500 μL 95% EtOH and adding this mixture to 50 mL 100 mM pH 10.4 borate buffer, add 100 μL 2-mercaptoethanol.)

HPLC VARIABLES

Guard column: 37-50 μm Bondapak C18/Corasil
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was THF:water 3:97 containing 100 mM potassium phosphate, pH 7.0. B was THF:MeCN:water 3:40:57 containing 100 mM potassium phosphate, pH 7.0. A:B 97:3 for 1.5 min, to 68:32 over 17 min (Waters curve profile 3), to 0:100 over 2 min, maintain at 0:100 for 4.5 min, return to initial conditions over 2 min, re-equilibrate for 8 min.
Column temperature: 41
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 455

CHROMATOGRAM

Retention time: 3.5 (aspartate, cysteate), 5 (cysteinesulfinate), 6 (glutamate), 10.5 (serine), 11 (glutamine), 12.5 (arginine), 13.5 (glycine), 15 (threonine, glycerophosphorylethanolamine), 15.3 (o-phosphorylethanolamine), 18.5 (alanine), 20.5 (hypotaurine), 21.5 (taurine), 22 (β- and gamma-aminobutyrate), 25 (tyrosine), 25.5 (α-aminobutyrate), 26 (methionine), 29 (ethanolamine)

KEY WORDS

plasma; human; rat; liver; kidney; heart; brain; derivatization

REFERENCE

Hirschberger,L.L.; De La Rosa,J.; Stipanuk,M.H. Determination of cysteinesulfinate, hypotaurine and taurine in physiological samples by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 303–313.

SAMPLE

Matrix: blood, tissue
Sample preparation: 250 μL Plasma + 5 μL 2 mM norleucine + 2 mL 10% trichloroacetic acid, mix, centrifuge at 10000 g for 10 min. Adjust the pH of the supernatant to 9.0 with KOH. Remove a 40 μL aliquot and add it to 40 μL 100 mM pH 8.3 sodium bicarbonate, add 80 μL 4 mM dabsyl chloride in MeCN, heat at 70° for 12 min (mix after 1 and 4 min), cool to room temperature for 5 min, add 440 μL EtOH:50 mM pH 7.0 sodium phosphate buffer 50:50, inject a 50 μL aliquot. Liver. Homogenize (Polytron) 200 mg liver with 5 volumes of 10% trichloroacetic acid, mix, centrifuge at 10000 g for 10 min. Adjust the pH of the supernatant to 9.0 with KOH. Remove a 40 μL aliquot and add it to 40 μL 100 mM pH 8.3 sodium bicarbonate, add 80 μL 4 mM dabsyl chloride in MeCN, heat at 70° for 12 min (mix after 1 and 4 min), cool to room temperature for 5 min, add 440 μL EtOH: 50 mM pH 7.0 sodium phosphate buffer 50:50, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Adsorbosphere C18
Column: 250 × 4.6 5 μm Econosphere
Mobile phase: Gradient. A was DMF:10 mM pH 6.5 citrate buffer 4:96. B was MeCN:10 mM pH 6.5 citrate buffer:DMF 67.2:28.8:4. A:B 83:17 for 5 min, to 74:26 over 18.6 min, to 63:37 over 16 min, to 19:81 over 34.7 min, to 0:100 over 0.3 min, maintain at 0:100 for 1.4 min, return to initial conditions, re-equilibrate for 14 min. (Condition column with DMF:100 mM pH 3.5 sodium citrate 20:80 at 1 mL/min for 1 h before use.)
Flow rate: 1.4
Injection volume: 50

Detector: UV 436

CHROMATOGRAM

Retention time: 28.7 (aspartate), 30.9 (cysteine), 32.7 (glutamate), 43.4 (glutamine), 43.8 (serine), 44.6 (threonine), 45.3 (arginine), 46.0 (glycine), 46.5 (alanine), 48.6 (proline), 49.5 (valine), 51.0 (methionine), 51.5 (leucine), 52.2 (isoleucine), 54 (phenylalanine), 66.4 (lysine), 67.6 (histidine), 71.5 (tyrosine)
Internal standard: norleucine (53)

KEY WORDS

derivatization; rat; plasma; liver

REFERENCE

Drnevich,D.; Vary,T.C. Analysis of physiological amino acids using dabsyl derivatization and reversed-phase liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 137–144.

SAMPLE

Matrix: blood, tissue
Sample preparation: Serum. 10 μL Serum + 90 μL MeOH, mix vigorously, centrifuge at 1000 g for 5 min. Remove a 10 μL aliquot of the supernatant and add it to 10 μL 200 mM pH 8.0 borate buffer containing 4 mM tetrasodium EDTA, add 30 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, cool, add 250 μL MeOH:acetic acid 99:1, filter (0.5 μm), inject a 10 μL aliquot of the filtrate. Rat brain. Homogenize (glass-Potter) rat brain tissue in 10 volume MeOH at 4°, centrifuge at 1000 g for 10 min. Remove a 10 μL aliquot of the supernatant and add it to 10 μL 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA, add 30 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, cool, add 250 μL MeOH:acetic acid 99:1, filter (0.5 μm), inject a 10 μL aliquot of the filtrate. Cow brain. Homogenize (glass-Potter) rat brain tissue in 10 volume buffer at 4°, filter through gauze. Vigorously mix 10 μL filtrate with 90 μL MeOH, centrifuge at 1000 g for 5 min. Remove a 10 μL aliquot of the supernatant and add it to 10 μL 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA, add 30 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, cool, add 250 μL MeOH:acetic acid 99:1, filter (0.5 μm), inject a 10 μL aliquot of the filtrate. (Buffer was 10 mM pH 7.4 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) containing 122 mM NaCl, 3 mM KCl, 25 mM sodium bicarbonate, 1.4 mM calcium chloride, 1.2 mM magnesium sulfate, and 0.4 mM glucose.)

HPLC VARIABLES

Guard column: Resolve C18 guard column (Waters)
Column: 250 × 4.6 Sumichiral OA-3100(S) (Sumika)
Mobile phase: MeOH containing 5 mM citric acid
Flow rate: 1 (standards) or 0.5 (biological samples)
Injection volume: 10
Detector: F ex 470 em 530

CHROMATOGRAM

Retention time: k' 1.01 (first (D) enantiomer, α = 1.52 (Leu)), k' 1.18 (first (D) enantiomer, α = 1.25 (Ile)), k' 1.29 (first (D) enantiomer, α = 1.22 (Val)), k' 2.05 (first (D) enantiomer, α = 1.31 (Ala)), k' 5.48 (first (D) enantiomer, α = 1.06 (Pro)), k' 2.13 (first (D) enantiomer, α = 1.35 (Thr)), k' 3.10 (first (D) enantiomer, α = 1.33 (Ser)), k' 2.16 (first (D) enantiomer, α = 1.36 (Phe)), k' 2.08 (first (D) enantiomer, α = 1.40 (Met)), k' 3.00 (first (D) enantiomer, α = 1.27 (Gln)), k' 4.96 (first (D) enantiomer, α = 1.57 (Lys))

KEY WORDS

derivatization; serum; human; rat; cow; brain; chiral; detailed discussion of use of other chiral columns; detailed discussion of use of other derivatizing reagents

REFERENCE

Fukushima,T.; Kato,M.; Santa,T.; Imai,K. Enantiomeric separation and sensitive determination of D,L-amino acids derivatized with fluorogenic benzofurazan reagents on Pirkle type stationary phases, *Biomed.Chromatogr.*, **1995**, *9*, 10–17.

SAMPLE

Matrix: blood, tissue, urine, ascitic fluid

Sample preparation: Homogenize liver with 5 volumes ice-cold 3% sulfosalicylic acid, centrifuge at 2000 g for 15 min. Add an equal volume of cold 6% sulfosalicylic acid to plasma, urine, or ascitic fluid, mix, centrifuge at 4° at 1800 g for 12 min. Dilute the supernatant or liver homogenate with an equal volume of 400 μM methionine sulfone in 100 mM HCl, evaporate an aliquot to dryness under reduced pressure, add 20 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, reconstitute with 20 μL EtOH:triethylamine:water:phenyl isothiocyanate 70:10:10:10 (freshly prepared), let stand at room temperature for 20 min, evaporate to dryness under reduced pressure (70 mTorr) for 1.5-2 h, reconstitute with 250 μL pH 7.4 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Pico-Tag C18 (Waters)

Mobile phase: Gradient. A was MeCN:buffer 6:94. B was MeCN:water 60:40. A:B from 98:2 to 54:46 over 10 min (Waters convex curve No. 5), to 0:100 over 0.5 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 7 min. (Buffer was 140 mM sodium acetate in water containing 500 μL/L triethylamine, pH adjusted to 6.40 with glacial acetic acid.)

Column temperature: 38

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 1.7 (Asp), 1.9 (Glu), 2.7 (Hpro), 3.45 (Asn), 3.65 (Ser), 3.8 (Gln), 4.0 (Gly), 4.6 (His), 5.25 (Tau), 5.5 (Arg), 5.7 (Thr), 5.9 (Ala), 6.3 (Pro), 7.2 (AAB), 7.8 (Tyr), 8.4 (Val), 8.8 (Met), 9.5 (Cys), 9.75 (Ile), 9.9 (Leu), 10.9 (Phe), 11.3 (Trp), 12 (Lys)

Internal standard: methionine sulfone (6.5)

Limit of detection: 3 μM

KEY WORDS

derivatization; plasma; liver

REFERENCE

Fierabracci,V.; Masiello,P.; Novelli,M.; Bergamini,E. Application of amino acid analysis by high-performance liquid chromatography with phenyl isothiocyanate derivatization to the rapid determination of free amino acids in biological samples, *J.Chromatogr.*, **1991**, *570*, 285–291.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 100 μL Serum + 400 μL EtOH, mix, centrifuge. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute with 200 μL MeOH:triethylamine 95:5 (prepare fresh each day), add 10 μL phenylisothiocyanate:MeOH 12.5:87.5 (prepare fresh each day), mix, let stand at room temperature for 5 min, evaporate to dryness under reduced pressure, reconstitute with 500 μL buffer, add 200 μL dichloromethane, vortex for 1 min, centrifuge at 1200 g for 1-3 min, inject an aliquot of the aqueous layer. Urine. Add 10 μL IS solution to 50/x μL urine (x = concentration of creatinine (mM)), add 200 μL EtOH, mix, centrifuge. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute with 200 μL MeOH:triethylamine 95:5 (prepare fresh each day), add 10 μL phenylisothiocyanate:MeOH 12.5:87.5 (prepare fresh each day), mix, let stand at room temperature for 5 min, evaporate to dryness under reduced pressure, reconstitute with 500 μL buffer, add 200 μL dichloromethane, vortex

for 1 min, centrifuge at 1200 g for 1-3 min, inject an aliquot of the aqueous layer. (Prepare buffer by dissolving 1.36 g sodium acetate trihydrate in water, adjust pH to 6.40 ± 0.01 with 2% orthophosphoric acid, make up to 1 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil-ODS

Mobile phase: Gradient. A was 1.36 g/L sodium acetate trihydrate in water, pH adjusted to 6.40 ± 0.01 with 2% orthophosphoric acid. B was 1.36 g/L sodium acetate trihydrate in MeCN:water 60:40, pH adjusted to 6.40 ± 0.01 with 2% orthophosphoric acid. A:B from 100:0 to 87:13 over 20 min, to 45:55 over 45 min, to 0:100 over 2.5 min, maintain at 0:100 for 2.5 min, return to initial conditions over 5 min, re-equilibrate for 5 min.

Flow rate: 1 for 65 min then 2

Injection volume: 20

Detector: E, EDT Research LCA 15, glassy carbon electrode +1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 3.82 (phosphoserine), 5.44 (aspartic acid), 6.83 (glutamic acid), 10.35 (gamma-aminoadipic acid), 12.26 (hydroxyproline), 12.45 (phosphoethanolamine), 14.52 (serine), 15.52 (glycine), 15.54 (asparagine), 16.91 (sarcosine), 17.42 (β-alanine), 18.98 (taurine), 20.48 (gamma-aminobutyric acid), 20.63 (citrulline), 21.11 (threonine), 21.75 (alanine), 22.27 (β-aminoisobutyric acid), 23.48 (proline), 23.91 (histidine), 25.71 (carnosine), 28.22 (arginine), 28.39 (1-methylhistidine, 3-methylhistidine), 28.60 (α-aminobutyric acid), 29.06 (anserine), 35.02 (tyrosine), 35.76 (valine), 37.65 (ethanolamine), 38.10 (methionine), 38.19 (cystathionine), 40.88 (cystine), 42.80 (isoleucine), 43.47 (leucine), 47.78 (hydroxylysine), 48.20 (phenylalanine), 48.46 (hydroxylysine), 49.67 (ornithine), 50.16 (tryptophan), 52.79 (lysine)

Internal standard: norleucine (45.07)

Limit of quantitation: 1 μM

KEY WORDS

derivatization; serum

REFERENCE

Sherwood,R.A.; Titheradge,A.C.; Richards,D.A. Measurement of plasma and urine amino acids by high-performance liquid chromatography with electrochemical detection using phenylisothiocyanate derivatization, *J.Chromatogr.*, **1990**, *528*, 293–303.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 400 μL Plasma + 100 μL 100 mM dithiothreitol, heat at 37° for 15 min, add 100 μL 150 mg/mL sulfosalicylic acid, mix, let stand at room temperature for 30 min, centrifuge at 4000 g for 15 min, inject a 30 μL aliquot. Urine. 400 μL Urine + 30 μL 100 mM dithiothreitol + 70 μL 300 mM pH 8.5 Tris buffer, mix, heat at 37° for 15 min, add 100 μL 100 mg/mL sulfosalicylic acid, mix, let stand at room temperature for 30 min, centrifuge at 4000 g for 15 min, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3.2 7 μm G18-013 C18 (Brownlee)

Column: 100 × 3.2 3.2 Velosep RP-18 (Brownlee, Applied Biosystems)

Mobile phase: MeOH:buffer 4.3:100 (Buffer was 16 mM NaH_2PO_4, 19 mM phosphoric acid, and 8 mM octyl sulfate, pH 2.36 ± 0.02. Use a 30 × 3.2 5 μm Brownlee SS-GU silica column before the injector.)

Flow rate: 0.8

Injection volume: 30

Detector: UV 324 following post-column reaction. The effluent from the column mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 2 m × 0.5 mm stainless steel coil to the detector. (Prepare reagent by mixing (at 4°) 3 mL 10 mM 4,4'-

dithiopyridine in MeOH:10 mM HCl 3:97 with 300 mL 300 mM Tris base containing 1 mM EDTA (adjusted to pH 8.5 with phosphoric acid). Sparge with helium before use, keep in an ice bath during use.)

CHROMATOGRAM

Retention time: 5.4 (cysteine), 7.2 (glutathione), 10.4 (gamma-glutamylcysteine), 13.2 (cysteinylglycine), 15.9 (homocysteine)

Limit of detection: 50 nM

KEY WORDS

derivatization; plasma; post-column reaction

REFERENCE

Andersson,A.; Isaksson,A.; Brattström,L.; Hultberg,B. Homocysteine and other thiols determined in plasma by HPLC and thiol-specific postcolumn derivatization, *Clin.Chem.*, **1993**, *39*, 1590–1597.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 0.5 mmole amino acid and 0.5 mmole (1S)-(+)-10-camphorsulfonyl chloride in 10 mL ether, add 10 mL 1 M NaOH, stir vigorously for 1 h, acidify with 1 M HCl, extract with ether. Evaporate the extract to dryness and reconstitute with chloroform containing 0.5 mmole p-nitrobenzyl bromide, reflux for 30 min, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness.

HPLC VARIABLES

Column: 250 × 2 MicroPak Si-5

Mobile phase: Isooctane:isopropanol 98.5:1.5

Flow rate: 0.5

Detector: UV 254

CHROMATOGRAM

Retention time: 7.0 (L-isoleucine), 7.6 (D-isoleucine, L-leucine), 8.6 (D-leucine), 11.0 (L-phenylalanine), 12.4 (D-phenylalanine), 17.0 (L-alanine), 22.3 (D-alanine)

KEY WORDS

derivatization; normal phase; chiral

REFERENCE

Furukawa,H.; Sakakibara,E.; Kamei,A.; Ito,K. Separation of L- and D-amino acids as diastereomeric derivatives by high performance liquid chromatography, *Chem.Pharm.Bull.*, **1975**, *23*, 1625–1626.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1 mmole amino acid in 10 mL diethyl ether, add 20 mL 1 M NaOH, stir vigorously at 0°, add 2 mmole (1S)-(+)-10-camphorsulfonyl chloride in 30 mL ether dropwise, stir at room temperature for 1 h. Remove the aqueous layer and wash it twice with diethyl ether, acidify the aqueous layer with concentrated HCl, extract with diethyl ether. Dry the organic extract over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 10 mL DMF, add 1 drop trimethylamine, add 1.1 mmole p-nitrobenzyl bromide, heat at 55° for 2 h, dilute with 40 mL chloroform, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with chloroform, inject an aliquot.

HPLC VARIABLES

Column: 250 × 2.2 10 μm MicroPak-NH2

Mobile phase: Isooctane:dichloromethane:isopropanol 79:16:5

Flow rate: 0.4

Detector: UV 254

CHROMATOGRAM

Retention time: 3.9 (L-leucine), 4.4 (L-isoleucine, D-leucine), 5.0 (D-isoleucine), 6.2 (L-phenylalanine), 7.2 (L-alanine), 7.4 (L-methionine), 9.3 (D-alanine), 8.5 (D-phenylalanine), 10.0 (D-methionine), 12.8 (L-glutamic acid), 16.8 (D-glutamic acid), 29.2 (L-tryptophan), 33.2 (L-tyrosine), 47.2 (D-tyrosine), 49.6 (D-tryptophan)

KEY WORDS

derivatization; normal phase; chiral

REFERENCE

Furukawa,H.; Mori,Y.; Takeuchi,Y.; Ito,K. Separation of L- and D-amino acids as diastereomeric derivatives by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *136*, 428–431.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1 mg amino acids in 100 μL 100 mM pH 9.0 sodium bicarbonate buffer, add 100 μL 2 mM dabsyl chloride in acetone, if necessary adjust pH to 9.0 with 100 mM NaOH, heat at 70° for 10 min, evaporate to dryness under reduced pressure, reconstitute with EtOH:water 70:30, inject a 5-25 μL aliquot. (Purify dabsyl chloride by dissolving 1 g in 100 mL boiling acetone, filtering (sintered glass), cooling to -20° overnight, and collecting the needle-shaped crystals.)

HPLC VARIABLES

Column: Zorbax ODS
Mobile phase: Gradient. MeCN:buffer from 20:80 to 70:30 over 25 min, maintain at 70:30.
Flow rate: 1.2
Injection volume: 5-25
Detector: UV 436

CHROMATOGRAM

Retention time: 10 (cysteic acid), 12 (carboxymethylcysteine), 13 (Asp, Ser), 14 (Glu), 14.5 (Thr), 15 (Gly), 16 (Arg), 17 (Ala), 19 (Met), 20 (Pro), 20.5 (Val), 21.5 (Phe), 22 (ammonia), 23 (Leu, Ile), 26.5 (His), 27.5 (Lys), 30 (Tyr)
Limit of detection: 2-5 pmole

KEY WORDS

derivatization

REFERENCE

Chang,J.-Y.; Knecht,R.; Braun,D.G. Amino acid analysis at the picomole level. Application to the C-terminal sequence analysis of polypeptides, *Biochem.J.*, **1981**, *199*, 547–555.

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 5 mg/mL solution in 1 M HCl or MeOH:water 25:75 depending on solubility, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 mm long μBondapak C18
Mobile phase: 8 mM (S)-Proline containing 4 mM cupric acetate, adjusted to pH 5 with NaOH
Flow rate: 3
Injection volume: 20
Detector: F ex 365 em 455 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min and this mixture flowed to the detector. (Prepare the

reagent by adding 7.5 mL 1% o-phthalaldehyde in EtOH:water 95:5, 1.8 mL Brij-35 surfactant, 200 μL mercaptoethanol, and 570 mg EDTA to 285 mL 50 mM pH 9.5 boric acid buffer, adjust pH to 10-11 with NaOH.)

CHROMATOGRAM
Retention time: 4.54 (R-valine), 10.03 (S-valine), 11.00 (R-tyrosine), 20.24 (S-tyrosine)
Limit of detection: 0.01% (of major enantiomer): chiral

KEY WORDS
derivatization; post-column reaction

REFERENCE
Cotter,M.L.; Naldi,R.; Shaw,C.; Park,S.; Heavner,G.A. Detection and quantitation of low levels of protected and unprotected (*R*)-amino acids in the synthesis of thymopentin, an immunoregulatory peptide, *J.Pharm.Sci.*, **1985**, *74*, 489–491.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 500 μg amino acids in 1 mL pH 10.65 triethylamine/acetic acid buffer, remove a 100 μL aliquot and add it to 50 μL 4 mM 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate in acetone, heat at 54° for 1 h, dry under vacuum, dissolve the residue in 100 μL 50% trifluoroacetic acid, heat at 54° for 45 min, dry under vacuum, reconstitute with EtOH:water, inject a 20 μL aliquot. (Purify 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate by dissolving 500 mg in 50 mL boiling acetone, filter (sintered glass), store the filtrate at -20° overnight to obtain 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate as needle-shaped crystals.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pellicular Packing LC-18
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: Gradient. MeCN:35 mM pH 5.1 sodium acetate buffer 39:61 for 8 min, to 53:47 over 4 min, maintain at 53:47 for 28 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 436

CHROMATOGRAM
Retention time: 5 (cysteic acid), 6 (aspartic acid), 7 (carboxymethylcysteine), 5 (glutamic acid), 10.8 (glutamine), 12 (glutamine), 12.8 (asparagine), 13.2 (serine), 14 (threonine), 16.4 (glycine), 17.2 (histidine), 18.8 (tyrosine), 19.6 (alanine), 17.2 (methionine), 18 (tryptophan), 18.8 (valine), 19.6 (proline), 32.4 (phenylalanine), 37.2 (isoleucine), 38.4 (leucine)
Limit of detection: <1 pmole

KEY WORDS
derivatization

REFERENCE
Stocchi,V.; Cucchiarini,L.; Piccoli,G.; Magnani,M. Complete high-performance liquid chromatographic separation of 4-N,N-dimethylaminoazobenzene-4'-thiohydantoin and 4-dimethylaminoazobenzene-4'-sulphonyl chloride amino acids utilizing the same reversed-phase column at room temperature, *J.Chromatogr.*, **1985**, *349*, 77–82.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 250 nmoles of the amino acid in 500 μL 50 mM pH 10.0 sodium borate buffer, add 500 μL 6 mM 4-N,N-dimethylamino-1-naphthyl isothiocyanate

in dioxane (Caution! Dioxane is a carcinogen!), purge with nitrogen, heat at 40° for 1.5 min, concentrate under a stream of nitrogen, add 500 μL water, add 1 mL hexane, vortex, centrifuge, discard the hexane layer, repeat the hexane wash twice more. Dry the aqueous layer under a stream of nitrogen, reconstitute with 500 μL trifluoroacetic acid:water 50:50, purge with nitrogen, heat at 80° for 10 min, dry under a stream of nitrogen, reconstitute with 1 mL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-120A (Toyo Soda, Tokyo)
Mobile phase: Gradient. A was MeCN:50 mM pH 7.0 imidazole nitrate buffer 15:85. B was MeCN:water 60:40. A:B from 100:0 to 0:100 over 25 min.
Flow rate: 1
Injection volume: 10
Detector: F ex 345 em 435 following post-column reaction. The column effluent mixed with MeCN:150 mM NaOH 60:40 pumped at 1 mL/min and the mixture flowed through a 50 cm × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM

Retention time: 12.1, 12.4 (Asp), 13.6 (Glu), 13.6, 14.1 (carboxymethylcysteine), 17.4 (Asn), 17.6, 18.7 (His), 18.0, 18.4 (Ser), 18.5 (Gln), 18.6, 19.0 (Thr), 20.3, 20.9 (Arg), 20.6 (Gly), 21.8, 23.0 (Tyr), 22.7 (Ala), 25.9, 27.2 (Trp), 26.2, 26.6 (Met), 26.7 (Val), 27.1, 31.5 (Lys), 27.8, 28.5 (Phe), 28.7 (Ile), 29.0 (Leu)
Limit of detection: 0.2 pmole

KEY WORDS

derivatization; post-column reaction; stereoisomers give rise to two peaks for some amino acids

REFERENCE

Miyano,H.; Nakajima,T.; Imai,K. Micro-scale sequence analysis from the N-terminus of peptides using the fluorogenic Edman reagent 4-*N,N*-dimethylamino-1-naphthyl isothiocyanate, *Biomed.Chromatogr.*, **1987**, *2*, 139–144.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:67 mM pH 7.0 phosphate buffer 65:27:8 (A) or 70:25:5 (B) or 80:15:5 (C)
Flow rate: 0.42 (A) or 0.45 (B) or 0.50 (C)
Injection volume: 10
Detector: UV 231

CHROMATOGRAM

Retention time: k' 5.19 (D-proline (B)), k' 5.35 (L-threonine (B)), k' 6.22 (L-tyrosine (B)), k' 6.24 (D-threonine (B)), k' 6.24 (L-2-aminobutyric acid (B)), k' 6.38 (L-phenylglycine (B)), k' 6.41 (L-proline (B)), k' 7.22 (L-valine (B)), k' 7.37 (L-penicillamine (B)), k' 7.41 (D-tyrosine (B)), k' 7.57 (D-2-aminobutyric acid (B)), k' 7.86 (D-phenylglycine (B)), k' 8.08 (L-methionine (B)), k' 9.16 (D-valine (B)), k' 9.27 (L-isoleucine (B)), k' 9.43 (L-tryptophan (B)), k' 9.51 (L-leucine (B)), k' 10.05 (D-penicillamine (B)), k' 10.24 (D-methionine (B)), k' 10.54 (L-phenylalanine (B)), k' 11.94 (L-ornithine (C)), k' 12.03 (D-tryptophan (B)), k' 12.35 (D-isoleucine (B)), k' 12.65 (D-leucine (B)), k' 12.89 (L-norleucine (B)), k' 13.48 (L-

lysine (C)), k' 13.81 (D-phenylalanine (B)), k' 13.90 (D-ornithine (C)), k' 15.32 (D-lysine (C)), k' 16.81 (D-norleucine (B)), k' 16.88 (L-3-aminobutyric acid (A)), k' 18.00 (L-alanine (A)), k' 18.95 (D-3-aminobutyric acid (A)), k' 20.85 (D-alanine (A))

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve amino acids in 70 μL AccQ.Fluor Borate Buffer (Waters), add 20 μL AccQ.Fluor Reagent (10 mM 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in MeCN (Waters)), vortex, let stand for 1 h at room temperature, heat at 55° for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Chiradex (immobilized β-cyclodextrin) (Merck)
Mobile phase: MeOH:100 mM pH 6.5 ammonium acetate buffer containing 0.1% triethylamine 50:50
Column temperature: 20 ± 0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 254 or F ex 250 em 395

CHROMATOGRAM
Retention time: k' 2.45 (second enantiomer, α = 1.08 (Ala)), k' 2.15 (second enantiomer, α = 1.00 (Val)), k' 4.89 (second enantiomer, α = 1.39 (Leu)), k' 3.16 (second enantiomer, α = 1.13 (Ile)), k' 2.51 (second enantiomer, α = 1.10 (Pro)), k' 2.90 (second enantiomer, α = 1.08 (Met)), k' 2.40 (second enantiomer, α = 1.08 (Cys)), k' 2.50 (second enantiomer, α = 1.07 (Ser)), k' 2.29 (second enantiomer, α = 1.07 (Thr)), k' 4.86 (second enantiomer, α = 1.16 (Lys)), k' 1.44 (second enantiomer, α = 1.13 (Arg)), k' 2.46 (second enantiomer, α = 1.11 (Asn)), k' 2.05 (second enantiomer, α = 1.06 (Gln)), k' 16.64 (second enantiomer, α = 1.20 (Asp)), k' 10.08 (second enantiomer, α = 1.00 (Glu)), k' 7.06 (second enantiomer, α = 1.13 (Phe)), k' 4.82 (second enantiomer, α = 1.04 (Trp)), k' 2.50 (second enantiomer, α = 1.06 (His)), k' 5.19 (second enantiomer, α = 1.25 (Tyr))

KEY WORDS
chiral; derivatization; α = k' (second enantiomer)/k' (first enantiomer); detailed comparison with other derivatizing reagents

REFERENCE
Rizzi,A.M.; Cladrowa-Runge,S.; Jonsson,H.; Osla,S. Enantiomeric resolution of derivatized DL-amino acids by high-performance liquid chromatography using a β-cyclodextrin chiral stationary phase: A comparison between derivatization labels, *J.Chromatogr.A*, **1995**, *710*, 287–295.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 460 μL 100 mM boric acid adjusted to pH 9 with NaOH, add 300 μL 100 mM dansyl chloride in acetone, let stand in dark for 2 h, evaporate to dryness under reduced pressure, reconstitute with 500 μL acetone:1 M HCl 95:5, centrifuge for 5 min, evaporate the liquid to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Chiradex (immobilized β-cyclodextrin) (Merck)
Mobile phase: MeOH:100 mM pH 5.5 ammonium acetate buffer containing 0.1% triethylamine 70:30
Column temperature: 20 ± 0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 254, F ex 325 em 350

CHROMATOGRAM

Retention time: k' 2.52 (second enantiomer, α = 1.08 (Ala)), k' 2.03 (second enantiomer, α = 1.22 (Val)), k' 2.54 (second enantiomer, α = 1.75 (Leu)), k' 1.68 (second enantiomer, α = 1.32 (Ile)), k' 1.40 (second enantiomer, α = 1.10 (Pro)), k' 1.33 (second enantiomer, α = 1.24 (Met)), k' 2.99 (second enantiomer, α = 1.15 (Cys)), k' 1.37 (second enantiomer, α = 1.16 (Ser)), k' 1.46 (second enantiomer, α = 1.29 (Thr)), k' 1.22 (second enantiomer, α = 1.10 (Lys)), k' 0.84 (second enantiomer, α = 1.16 (Arg)), k' 1.68 (second enantiomer, α = 1.11 (Asn)), k' 1.38 (second enantiomer, α = 1.14 (Gln)), k' 18.03 (second enantiomer, α = 1.13 (Asp)), k' 9.17 (second enantiomer, α = 1.15 (Glu)), k' 2.42 (second enantiomer, α = 1.43 (Phe)), k' 1.63 (second enantiomer, α = 1.00 (Trp)), k' 1.66 (second enantiomer, α = 1.07 (His)), k' 2.67 (second enantiomer, α = 1.25 (Tyr))

KEY WORDS

chiral; derivatization; α = k' (second enantiomer)/k' (first enantiomer); detailed comparison with other derivatizing reagents

REFERENCE

Rizzi,A.M.; Cladrowa-Runge,S.; Jonsson,H.; Osla,S. Enantiomeric resolution of derivatized DL-amino acids by high-performance liquid chromatography using a β-cyclodextrin chiral stationary phase: A comparison between derivatization labels, *J.Chromatogr.A*, **1995**, *710*, 287–295.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 1 mg amino acid in 250 μL water and 250 μL 40 mM pH 7.7 borate buffer, add 400 μL MeCN, add 3 mg reagent, vortex, let stand for about 30 min, extract with 3 mL ethyl acetate, inject an aliquot of the organic layer. (Synthesize the reagent, fluorenylmethoxycarbonyl glycyl chloride (FMOC-glycyl-Cl), as follows. Dissolve 1.0 g fluorenylmethoxycarbonyl glycine (FMOC glycine) in 15 mL dichloromethane, 3.4 mL thionyl chloride, reflux for 4 h, evaporate to dryness under reduced pressure, dissolve the residue in 1 mL dichloromethane, add 10 mL hexane. Filter the precipitate and dry it under vacuum at room temperature for 2 h to obtain fluorenylmethoxycarbonyl glycyl chloride.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm gamma-cyclodextrin Cyclobond II
Mobile phase: MeCN:triethylamine:acetic acid 100:1.2:0.3
Flow rate: 1
Detector: UV 265

CHROMATOGRAM

Retention time: k' 2.9 (methionine, α = 1.10), k' 3.0 (valine, α = 1.00), k' 3.1 (norleucine, α = 1.07), k' 3.1 (O-methyltyrosine, α = 1.10), k' 3.3 (norvaline, α = 1.09), k' 3.4 (phenylalanine, α = 1.11), k' 3.4 (alanine, α = 1.07), k' 3.6 (leucine, α = 1.15), k' 4.1 (homophenylalanine, α = 1.11), k' 8.3 (asparagine, α = 1.17), k' 8.66 (glutamine, α = 1.26), k' 12.1 (tryptophan, α = 1.29), k' 13.0 (aspartic acid, α = 1.06), k' 37.4 (glutamic acid, α = 1.21) (k' is the capacity factor of the first eluted enantiomer which is L except for aspartic acid, phenylalanine, and tryptophan.)

KEY WORDS

derivatization; chiral; comparison with other derivatizing reagents; details of chromatography with other mobile phases and with a β-cyclodextrin column are also given in the paper

REFERENCE

Tang,Y.; Zukowski,J.; Armstrong,D.W. Investigation on enantiomeric separations of fluorenylmethoxycarbonyl amino acids and peptides by high-performance liquid chromatography using native cyclodextrins as chiral stationary phases, *J.Chromatogr.A*, **1996**, *743*, 261–271.

SAMPLE

Matrix: bulk

Sample preparation: Treat 0.2-10 μmoles amino acids with 50-100 μL MeOH:thionyl chloride 95:5 at 60° for 2 h, evaporate to dryness, reconstitute with 35 μL 28% diisopropylethylamine in DMF, shake at room temperature for 10 min, add 25 μL 200-1000 mM N-α-(9-fluorenylmethyloxycarbonyl)leucine-N-carboxyanhydride in DMF, mix, let stand at room temperature for 10 min, add 200 μL 250 mM pH 8 sodium glycinate, mix, let stand for 5 min, add 300 μL chloroform, extract, dilute 10000-50000-fold with n-hexane, inject a 20 μL aliquot. (Synthesis of N-α-(9-fluorenylmethyloxycarbonyl)leucine-N-carboxyanhydride is as follows. Dry all solvents over 4 Å molecular sieve. Stir 20 mmoles L-leucine in 67 mL THF under nitrogen at 2° in an ice bath, add 22 mmoles 9-fluorenylmethyl chloroformate at once, slowly add 29 mmoles dry 4-methylmorpholine (N-methylmorpholine), stir at 2-5° for 2 h, slowly add 4 M HCl in dioxane (Caution! Dioxane is a carcinogen!) until the pH of a sample diluted with water reaches 4-5, filter, wash the solid with dry THF, concentrate the filtrate under reduced pressure, dissolve the resulting oil in the minimum volume of dry diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), add dry hexane until the solution just turns cloudy, let stand at -20° overnight, filter. Wash the solid with dry hexane and dry it under vacuum to obtain N-α-(9-fluorenylmethyloxycarbonyl)leucine-N-carboxyanhydride (mp 118-120°; $[\alpha]^{25}_{D}$ = +38.0°) (J. Am. Chem. Soc. 1990, 112, 7414).)

HPLC VARIABLES

Guard column: 15 × 3.2 5 μm Kromasil silica
Column: 250 × 4.6 5 μm Kromasil silica
Mobile phase: n-Hexane:isopropanol 98:2 (A) or 97:3 (B) or 95:5 (C) or 90:10 (D)
Flow rate: 0.8
Injection volume: 20
Detector: F ex 263 em 313

CHROMATOGRAM

Retention time: k' 1.35 (L-tyrosine (D)), k' 1.42 (L-tryptophan (D)), k' 1.66 (D-tyrosine (D)), k' 1.70 (L-threonine (D)), k' 1.72 (D-tryptophan (D)), k' 1.94 (D-threonine (D)), k' 1.97 (L-lysine (C)), k' 1.98 (L-leucine (A)), k' 2.11 (L-isoleucine (A)), k' 2.20 (L-valine (A)), k' 2.24 (L-2-aminohexanoic acid (A)), k' 2.37 (D-leucine (A)), k' 2.50 (L-β-(1-naphthyl)alanine (UV detection) (A)), k' 2.55 (L-phenylalanine (A)), k' 2.58 (L-2-amino-4-phenylbutyric acid (A)), k' 2.60 (D-isoleucine (A)), k' 2.60 (D-valine (A)), k' 2.60 (L-2-aminopentanoic acid (A)), k' 2.75 (D-2-aminohexanoic acid (A)), k' 2.87 (L-β-(2-naphthyl)alanine (UV detection) (A)), k' 2.88 (L-α-aminophenylacetic acid (A)), k' 2.94 (L-β-(2-thienyl)alanine (A)), k' 2.98 (L-β-(p-chlorophenyl)alanine (A)), k' 3.03 (D-lysine (C)), k' 3.12 (L-β-(3,4-dichlorophenyl)alanine (A)), k' 3.18 (D-2-aminopentanoic acid (A)), k' 3.27 (L-serine (C)), k' 3.35 (L-methionine (A)), k' 3.35 (D-2-amino-4-phenylbutyric acid (A)), k' 3.38 (L-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid), k' 3.42 (D-α-aminophenylacetic acid (A)), k' 3.51 (D-β-(1-naphthyl)alanine (UV detection) (A)), k' 3.59 (D-serine (C)), k' 3.61 (L-α-aminobutyric acid (A)), k' 4.11 (D-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (A)), k' 4.14 (D-β-(2-thienyl)alanine (A)), k' 4.34 (D-α-aminobutyric acid (A)), k' 4.42 (D-β-(2-naphthyl)alanine (UV detection) (A)), k' 4.71 (D-β-(p-chlorophenyl)alanine (A)), k' 4.88 (D-β-(3,4-dichlorophenyl)alanine (A)), k' 4.91 (D-methionine (A)), k' 4.91 (D-phenylalanine (A)), k' 5.04 (L-glutamic acid (B)), k' 5.37 (L-alanine (A)), k' 5.97 (D-alanine (A)), k' 6.24 (L-aspartic acid (B)), k' 7.35 (D-aspartic acid (B)), k' 7.8 (D-glutamic acid (B))

KEY WORDS
derivatization; normal phase; the use of related derivatization reagents is discussed; chiral

REFERENCE
Pugniere,M.; Mattras,H.; Castro,B.; Previero,A. Adsorption liquid chromatography on silica for the chiral separation of amino acids and asymmetric amines derivatized with optically active N-α-9-fluorenylmethyloxyxarbonyl-amino acid-N-carboxyanhydrides, *J.Chromatogr.A*, **1997**, *767*, 69–75.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge 350 μL cell suspensions at 1470 g for 1 min, add 20 μL 16% trichloroacetic acid to the supernatant, freeze. Thaw the supernatant and adjust its pH to 7.0 with 1 M NaOH, remove a 300 μL aliquot and make up to 400 μL with water, add 100 μL 1 M pH 6.2 boric acid, add 40 μL 10 μM dihydrokainic acid in MeCN:water 10:90, vortex for 10 s, add 500 μL 15 mM 9-fluorenylmethyl chloroformate, mix for 45 s, add 1 mL ethyl acetate, mix for 10 s, centrifuge at 10500 g for 2 min, repeat the ethyl acetate wash, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 C18 (Phenomenex)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN. B was 0.1% trifluoroacetic acid in water. A:B from 30:70 to 50:50 over 15 min, to 100:0 over 2 min, maintain at 100:0 for 5 min, return to initial conditions over 2 min, re-equilibrate for 12 min (cf. Int. J. Environ. Anal. Chem. 1990, 38, 351).
Column temperature: 55
Flow rate: 1
Injection volume: 25
Detector: F ex 264 em 313

CHROMATOGRAM
Retention time: 3.5 (taurine), 11 (glutamine), 12.5 (glutamate), 13 (aspartate)
Internal standard: dihydrokainic acid (21)

KEY WORDS
derivatization

REFERENCE
Brown,J.A.; Nijjar,M.S. The release of glutamate and aspartate from rat brain synaptosomes in response to domoic acid (amnesic shellfish toxin) and kainic acid, *Mol.Cell.Biochem.*, **1995**, *151*, 49–54.

SAMPLE
Matrix: cheese
Sample preparation: Homogenize (Ultra-Turrax T25) 16 g cheese with 30 mL water at room temperature for 2 min, centrifuge at 4° at 8650 g for 20 min, remove the water and fat layers. Homogenize the pellet in 30 mL water, centrifuge, remove the water and fat layers, repeat this process. Combine the fat layers and homogenize them with 20 mL water, centrifuge, remove the water layer. Combine all the water layers, filter (Whatman No. 42 paper) at 4°, filter (0.45 μm) at 4°, purify on a 200 × 4 Sephadex G-25 column with water at 84 mL/min with detection at UV 206, collect 84 mL fractions. Evaporate a 200 μL aliquot to dryness under reduced pressure, reconstitute with 200 μL 500 mM pH 7.8 borate buffer, add 200 μL 5.8 mM 9-fluorenylmethyl chloroformate in acetone, vortex for 45 s, wash with 400 μL pentane:ethyl acetate 80:20, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: Gradient. A was MeCN:100 mM pH 3.8 ammonium acetate buffer 20:80. B was MeCN:100 mM pH 4.2 ammonium acetate buffer 80:20. A:B 70:30 for 10 min, to 20:80 over 50 min, to 0:100 (step gradient), maintain at 0:100 for 10 min.
Column temperature: 40
Flow rate: 1
Detector: UV 214

CHROMATOGRAM

Retention time: 20 (arginine), 22.5 (serine, aspartic acid), 25 (glutamic acid), 27 (threonine), 30 (glycine), 35 (alanine), 40 (proline), 44 (valine), 46 (methionine), 51 (phenylalanine), 52.5 (isoleucine, leucine), 65 (histidine), 67.5 (lysine)

OTHER SUBSTANCES

Also analyzed: dipeptides, tripeptides

KEY WORDS

derivatization

REFERENCE

Roturier,J.M.; Le Bars,D.; Gripon,J.C. Separation and identification of hydrophilic peptides in dairy products using FMOC derivatization, *J.Chromatogr.A*, **1995**, *696*, 209–217.

SAMPLE

Matrix: chitin, protein, soil
Sample preparation: Hydrolyse 2.5 g sample with 90 mL boiling 6 M HCl for 6 h, filter, dilute filtrate to 100 mL, add 2 mL L-norleucine solution. Evaporate a 10 μL aliquot to dryness under reduced pressure, reconstitute with 20 μL 20 mM HCl, add 60 μL 200 mM pH 8.8 borate buffer, add 20 μL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters) in MeCN, heat at 40° for 5 min, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm AccQ-Tag C18 (Waters)
Mobile phase: Gradient. MeCN:water:buffer from 0:0:100 to 1:0:99 over 0.5 min, to 5:0:95 over 17.5 min, to 9:0:91 over 1 min, to 17:0:83 over 10.5 min, to 60:40:0 over 3.5 min, return to initial conditions over 22 min. (Buffer was 140 mM sodium acetate containing 17 mM triethylamine, adjusted to pH 5.05 with phosphoric acid.)
Column temperature: 37
Flow rate: 1
Injection volume: 5
Detector: F ex 250 em 395

CHROMATOGRAM

Retention time: 7.8 (β-Galactosamine), 18.9 (α-Galactosamine), 9.1 (β-Glucosamine), 15.9 (α-Glucosamine), 17.0 (Asp), 19.3 (Ser), 19.6 (Glu), 21.3 (Gly), 22.2 (His), 23.3 (ammonia), 25.8 (Arg), 26.2 (Thr), 26.9 (Ala), 28.2 (Pro), 29.3 (α-aminobutyric acid), 31.3 (Tyr), 32.4 (Val), 32.8 (Met), 35.7 (Lys), 36.1 (Ile), 36.5 (Leu), 38.3 (Phe)
Internal standard: L-norleucine (37.4)
Limit of detection: 49-780 fmole

KEY WORDS

derivatization

REFERENCE

Díaz,J.; Lliberia,J.L.; Comellas,L.; Broto-Puig,F. Amino acid and amino sugar determination by derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate followed by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.A*, **1996**, *719*, 171–179.

SAMPLE

Matrix: collagen

Sample preparation: Hydrolyze protein with 6 M HCl under nitrogen at 108° for 24 h, evaporate to dryness under reduced pressure, reconstitute with 100 mM sodium bicarbonate to give a hydrolysate concentration of 1 μg/mL. Remove a 5 μL aliquot and dilute it to 200 μL with 100 mM sodium bicarbonate, add 200 μL 4 mM fluorenylmethyl chloroformate in dry acetone, shake quickly, let stand at room temperature for 10 min, wash twice with 600 μL portions of pentane:ethyl acetate 90:10, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 MicroPak ODS-80TM

Mobile phase: Gradient. A was buffer. B was MeOH:buffer adjusted to pH 4.5 with phosphoric acid 20:80. C was MeCN. A:B:C 72.5:0:27.5 for 4.1 min, to 60:0:40 over 11.5 min, to 0:64:36 over 0.1 min, to 0:62:38 over 6.3 min, to 0:30:70 over 7 min, to 0:25:75 over 5 min, return to initial conditions, re-equilibrate for 11 min. (Buffer was 20 mM sodium citrate containing 5 mM tetramethylammonium chloride, pH 2.85.)

Column temperature: 30

Flow rate: 1.4

Injection volume: 20

Detector: F ex 254 em 340

CHROMATOGRAM

Retention time: 3.50 (histidine (mono-derivative)), 4.24 (cysteic acid), 4.78 (arginine), 7.60 (methionine sulfoxide), 8.92 (4-hydroxyproline), 9.80 (serine), 10.22 (3-hydroxyproline, homoserine), 10.80 (aspartic acid), 11.68 (glutamic acid), 12.48 (threonine), 13.05 (S-carboxymethylcysteine), 13.96 (glycine), 16.21 (ammonia), 16.60 (alanine), 17.48 (tyrosine (mono-derivative)), 18.30 (proline), 19.90 (methionine), 21.00 (valine), 23.27 (phenylalanine), 23.66 (isoleucine), 24.02 (leucine), 26.90 (cystine (bis-derivative)), 28.17 (hydroxylysine (bis-derivative), 28.81 (histidine (bis-derivative)), 30.00 (lysine (bis-derivative)), 31.85 (tyrosine (bis-derivative))

Limit of quantitation: 1 pmole

KEY WORDS

derivatization

REFERENCE

Miller,E.J.; Narkates,A.J.; Niemann,M.A. Amino acid analysis of collagen hydrolysates by reverse-phase high-performance liquid chromatography of 9-fluorenylmethyl chloroformate derivatives, *Anal.Biochem.*, **1990**, *190*, 92–97.

SAMPLE

Matrix: contact lenses

Sample preparation: Hydrolyze contact lens with 200 μL concentrated HCl at 145° for 1 h or with 200 μL 6 M HCl at 105° for 20 h, remove a 50-150 μL aliquot and evaporate it to dryness under reduced pressure, add 20 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 30 μL EtOH:water:triethylamine:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 25 min, reconstitute with 30-200 μL buffer, inject a 10 μL aliquot. (Buffer was MeCN:5 mM Na_2HPO_4 adjusted to pH 6.8 with 2% phosphoric acid.)

HPLC VARIABLES

Column: 150 × 4.6 Ultrasphere ODS C18

Mobile phase: Gradient. A was 700 μL triethylamine in 1 L 100 mM sodium acetate, adjusted to pH 5.5 with glacial acetic acid. B was MeCN:A:water 315:250:185. A:B 90:10 for 2 min, to 62:38 over 6 min, to 55:45 over 0.5 min, to 40:60 over 7 min, to 10:90 over 3 min, to 0:100 over 1 min, return to initial conditions over 8 min, re-equilibrate for 6 min. (Mobile phase A maintained at 60°, mobile phase B maintained at 40°.)

Column temperature: 43
Flow rate: 1
Injection volume: 10
Detector: UV 254

KEY WORDS
derivatization

REFERENCE
Yan,G.; Nyquist,G.; Caldwell,K.D.; Payor,R.; McCraw,E.C. Quantitation of total protein deposits on contact lenses by means of amino acid analysis, *Invest.Ophthalmol.Vis.Sci.*, **1993**, *34*, 1804–1813.

SAMPLE
Matrix: dialysate, tissue
Sample preparation: Homogenize (Kontes micro-ultrasonic cell disrupter) rat brain with 100 μL 50 mM ice-cold perchloric acid and 10 ng homoserine for 5 s, centrifuge at 4° at 13000 g for 5 min, filter (0.2 μm) the supernatant. Mix 25 μL of the filtrate from the tissue or dialysate (Ringer's) with 50 (tissue) or 12.5 (dialysate) μL working reagent, let stand for 2 min, inject an aliquot. (Prepare the reagent stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate, discard after 5 days. Prepare the working reagent by diluting 1 mL stock solution with 3 mL 100 mM sodium tetraborate, let stand for 24 h before use.)

HPLC VARIABLES
Column: 80 × 4.6 3 μm C18 HR-80 (ESA)
Mobile phase: MeOH:water 28:72 containing 100 mM Na_2HPO_4 and 0.13 mM disodium EDTA adjusted to pH 6.00 (tissue) or pH 6.40 (dialysate) with phosphoric acid. (Prepare by dissolving 14.2 g Na_2HPO_4 and 50 mg disodium EDTA in 720 mL water, add 280 mL MeOH, adjust pH. Recycle mobile phase.)
Flow rate: 1.2
Injection volume: 20
Detector: E, ESA Model 5100A coulometric, model 5011 dual electrode analytical cell preceded by a 0.2 μm carbon filter at -0.4 V and +0.6 V

CHROMATOGRAM
Retention time: 1.5 (Asp), 2 (Glu), 3 (Ser), 4 (Gln), 5.5 (Gly, Thr), 7 (Phenylethanolamine), 9 (Taurine), 10.5 (Ala), 14 (Tyr), 15 (GABA)
Internal standard: homoserine (3.5)
Limit of detection: 100-200 pg

KEY WORDS
rat; brain; derivatization

REFERENCE
Donzanti,B.A.; Yamamoto,B.K. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates, *Life Sci.*, **1988**, *43*, 913–922.

SAMPLE
Matrix: enzyme incubations
Sample preparation: 1 mL Enzyme incubation + 11 mL 155 μM DL-α-aminobutyric acid in buffer, mix, add a 2 mL aliquot to an 8 mL column of Sephadex G-25 (Pharmacia PD-10), elute with buffer, discard the first 6 mL eluate, collect the next 4 mL eluate. Add 3.2 μL diethyl ethoxymethylenemalonate to this fraction, shake at 50° for 50 min, inject a 15 μL aliquot. (Buffer was 1 M pH 9 sodium borate buffer containing 0.02% sodium azide.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. MeCN:25 mM pH 6 sodium acetate containing 0.02% sodium azide from 9:91 to 14:86 over 3 min, maintain at 14:86 for 10 min, to 31:69 over 17 min, maintain at 31:69 for 20 min. (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!)
Flow rate: 0.9
Injection volume: 15
Detector: UV 280

CHROMATOGRAM

Retention time: 17.7 (N-epsilon-(2-propenal)lysine), 35 (lysine), 44.8 (lysine dipeptide)
Internal standard: DL-α-aminobutyric acid (19)
Limit of detection: <1 pmole

KEY WORDS

derivatization

REFERENCE

Girón,J.; Alaiz,M.; Vioque,E. High-performance liquid chromatographic determination of *N*-epsilon-(2-propenal)lysine in biological samples after derivatization with diethylethoxymethylenemalonate, *Anal.Biochem.*, **1992**, *206*, 155–160.

SAMPLE

Matrix: feces, insects, plants
Sample preparation: Grind black cherry leaves, insect larvae, or feces to pass 40 mesh. Weigh out an amount containing 0.2-2 mg protein, add 2 mL 4 M methanesulfonic acid in water containing 0.2% 3-(2-aminoethyl)indole, freeze in dry ice-acetone, evacuate to 50 μTorr, flush with nitrogen, evacuate to 50 μTorr, flush with nitrogen, evacuate to 50 μTorr, hydrolyze at 115° for 22 h, add c-allylglycine, buffer with 100 mM pH 8.0 sodium borate, adjust to pH 8.0 with NaOH, make up to 12 mL, centrifuge. Remove a 1 mL aliquot and add it to 1 mL 6 mM 9-fluorenylmethylchloroformate in acetone, let stand for 15 min at room temperature, extract twice with 2 mL portions of n-pentane, filter (0.45 μm), discard the first few drops, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 end-capped ODS-80TM Aminotag (Varian)
Mobile phase: Gradient. A was MeCN:15 mM citric acid + 10 mM tetramethylammonium chloride adjusted to pH 1.85 with NaOH 27:73. B was MeCN:THF:15 mM citric acid + 10 mM tetramethylammonium chloride adjusted to pH 4.50 with NaOH 35:5:60. C was MeCN:THF:15 mM citric acid + 10 mM tetramethylammonium chloride adjusted to pH 4.50 with NaOH 62:13:25. A:B:C from 100:0:0 to 50:50:0 over 3 min, to 0:100:0 over 14 min (Waters concave gradient 7), to 0:96:4 over 6 min (Waters concave gradient 7), to 0:85:15 over 4 min, to 0:50:50 over 4 min, to 0:35:65 over 4 min, to 0:0:100 over 5 min. (Linear gradients except where shown).
Column temperature: 30
Flow rate: 1.4
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 7.5 (His), 9.5 (Arg), 11.5 (Asn), 11.5 (Gln), 13 (Ser), 14 (Asp), 15 (Glu), 15.5 (Thr), 17 (Gly), 20.5 (Ala), 22.5 (Tyr), 23 (Pro), 26 (Met), 27 (Val), 29.5 (Phe), 30.5 (Trp), 31.5 (Ile), 32 (Leu), 33 (cystine), 35.5 (di-His), 36.5 (Cys), 37.5 (Lys), 40 (di-Tyr)
Internal standard: c-allylglycine (25)
Limit of quantitation: 30000 nM

KEY WORDS

derivatization; protein; hydrolysis; plants; insects; feces; cherry; leaves; insect; larvae; feces; ASN and GLN co-elute

REFERENCE
Malmer,M.F.; Schroeder,L.A. Amino acid analysis by high-performance liquid chromatography with methanesulfonic acid hydrolysis and 9-fluorenylmethylchloroformate derivatization, *J.Chromatogr.*, **1990**, *514*, 227–239.

SAMPLE
Matrix: feed
Sample preparation: Heat 500 mg feed and 2 mL 6 M HCl containing 1% phenol at 105° for 24 h, neutralize with 100 mM NaOH, make up to 100 mL, filter (0.22 μm nylon). Mix 150 μL hydrolysate with 150 μL 30 mM sodium 1,2-naphthoquinone-4-sulfonate in 100 mM HCl and 150 μL 50 mM sodium borate containing 90 mM NaOH, heat at 65° for 5 min, add 60 μL 250 mM HCl, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb ODS 2
Mobile phase: Gradient. A was buffer. B was MeCN:buffer 50:50. A:B 100:0 for 15 min, to 94:6 over 5 min, maintain at 94:6 for 10 min, to 86:14 over 1 min, to 83:17 over 14 min, to 73:27 over 1 min, to 71:29 over 14 min, to 0:100 over 2 min, maintain at 0:100 for 8 min, return to initial conditions over 2 min, re-equilibrate for 3 min. (Buffer was 50 mM acetic acid containing 50 mM sodium acetate, pH 4.75.)
Column temperature: 50
Flow rate: 0.8
Injection volume: 50
Detector: UV 305

CHROMATOGRAM
Retention time: 10 (Cys), 12 (Asp), 14.5 (Ser), 21 (Gly), 22 (Glu), 24 (His), 26 (Thr), 29.5 (Pro), 30.5 (Arg), 33.5 (Ala), 41 (Tyr), 45 (Orn), 47.5 (Val), 48 (Met), 42.5 (Lys), 43.5 (Ile), 46 (Leu), 47 (Phe)
Limit of detection: 40-100 pmole

KEY WORDS
derivatization

REFERENCE
Saurina,J.; Hernńdez-Cassou,S. Chromatographic determination of amino acids by pre-column derivatization using 1,2-naphthoquinone-4-sulfonate as reagent, *J.Chromatogr.A*, **1996**, *740*, 21–30.

SAMPLE
Matrix: feed, food
Sample preparation: Add 2 mL chilled performic acid to 50-70 mg sample, let stand at 0° for 16 h, add 300 μL 48% HBr, let stand at 0° for 15 min, evaporate to dryness under reduced pressure at ≤60°, add 10 mL 6 M HCl, freeze in liquid nitrogen, seal tube under vacuum, heat at 110 ± 2° for 22 h, cool to room temperature, filter. Remove a 1-2 mL aliquot and evaporate it to dryness under reduced pressure at ≤50°, reconstitute with 2.5 mM IS solution and water so that the amino acid concentration is <13 mM and the IS concentration is 250 μM. Remove a 10 μL aliquot and add it to 70 μL 200 mM pH 8.8 borate buffer, vortex for 10 s, add 20 μL 3 mg/mL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters) in MeCN, vortex immediately, heat at 50° for 10 min, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm AccQ-Tag C18 (Waters)
Mobile phase: Gradient. A was 140 mM sodium acetate containing 17 mM triethylamine and 100 mg/L sodium azide (Caution! Sodium azide is carcinogenic and toxic! Do not discharge to the plumbing system!), pH adjusted to 4.95 with phosphoric acid. B MeCN: water:acetone 60:40:0.01. A:B from 100:0 to 92:8 over 17 min, to 83:17 over 4 min, to 73:

27 over 11 min, to 50:50 over 2 min, maintain at 50:50 for 1 min, to 0:100 over 2 min, return to initial conditions over 1 min, re-equilibrate for 7 min.
Column temperature: 47
Flow rate: 1
Injection volume: 4
Detector: UV 248

CHROMATOGRAM

Retention time: 7.5 (cysteic acid), 12.5 (Asp), 13.5 (Ser), 14.5 (Glu), 15 (Gly), 15.5 (His), 19 (Arg), 19.5 (Thr), 20.7 (methionine sulfone), 21 (Ala), 24 (Pro), 28 (Val), 32 (Lys), 33 (Ile), 33.5 (Leu), 34.5 (Phe)
Internal standard: α-aminobutyric acid (25)

KEY WORDS

derivatization; corn; shrimp

REFERENCE

Liu,H.J.; Chang,B.Y.; Yan,H.W.; Yu,F.H.; Liu,X.X. Determination of amino acids in food and feed by derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and reversed-phase liquid chromatographic separation, *J.AOAC Int.*, **1995**, *78*, 736–744.

SAMPLE

Matrix: fermentation solutions
Sample preparation: Wash 7.5 g broth on a 150 μm nylon mesh with ice-cold 0.8% NaCl, float cells in saline, wash cells with ice water, cool cells in ice, add 5 mL water, add 500 μL 1 M 4-morpholinepropanesulfonic acid buffer, sonicate for 15 s, shake for 30 s, sonicate for 15 s, filter 0.45 μm, filter (10 000 MW cutoff filter) while centrifuging. Add a 200 μL aliquot of the filtrate to a 15 × 8 column of quaternary ion-exchange resin, wash with 10 mL water, elute with 10 mL 1 M acetic acid, freeze dry, reconstitute with 200 μL water. Mix 20 μL solution with 5 μL reagent, let stand for 2-3 min, add 475 μL 50 mM pH 5.2 sodium acetate (Anal.Biochem. 1984, 137, 405), filter (0.45 μm), inject an aliquot of the filtrate. (Prepare reagent by dissolving 4 mg o-phthaldialdehyde in 300 μL MeOH, add 250 μL 400 mM pH 8.0 borate buffer, add 390 μL water, add 60 μL 1 M N-acetyl-L-cysteine (adjusted to pH 5.0-6.0 with NaOH). Store at 4°, discard after 3 weeks (Anal.Biochem. 1984, 137, 405).)

HPLC VARIABLES

Column: 150 × 5 3 μm Hypersil ODS
Mobile phase: Gradient. A was 50 mM pH 5.9 sodium acetate. B was MeOH:50 mM pH 5.9 sodium acetate 80:20. A:B from 100:0 to 0:100 over 50 min (?).
Flow rate: 1
Detector: F ex 330-375 em 418 (cut-off filter)

CHROMATOGRAM

Retention time: 3.5 (glutathione), 4 (L-Asp), 4.5 (L-Glu), 5.8 (L-Ser), 9.5 (L-α-aminoadipic acid), 10.5 (D-α-aminoadipic acid), 11 (L-His), 11.5 (L-Thr), 12 (Gly), 19 (L-Arg), 22.5 (A-Ala), 27 (cephalosporin C), 28 (L-Tyr), 28 (penicillin N), 28.5 (isopenicillin N), 32.5 (L-Val), 34.5 (L-Met), 35 (D-Val), 39 (L-Phe), 40 (L-Ile), 47.5 (L-Leu), 49 (L-Lys)

OTHER SUBSTANCES

Noninterfering: L-cysteine

KEY WORDS

SPE; derivatization; chiral

REFERENCE

Usher,J.J.; Lewis,M.; Hughes,D.W. Determination by high-performance liquid chromatography of some compounds involved in the biosynthesis of penicillin and cephalosporin, *Anal.Biochem.*, **1985**, *149*, 105–110.

SAMPLE

Matrix: fermentation solutions

Sample preparation: Centrifuge 1 mL fermentation medium at 15000 g for 3 min, dilute the supernatant 10-100-fold with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.3 5 μm Separon C18 glass column (Tessek, Prague)

Mobile phase: MeOH:water 25:75 containing 1 mM copper sulfate

Column temperature: 45

Flow rate: 0.5

Injection volume: 1

Detector: UV 245

CHROMATOGRAM

Retention time: 0.5 (alanine), 0.6 (α-aminobutyric acid), 0.9 (valine), 1.65 (leucine), 3.5 (homoleucine)

KEY WORDS

derivatization; complexation

REFERENCE

Polanuer,B.M.; Ivanov,S.V. High-performance liquid chromatography of amino acids in copper(II) complex form: application to valine fermentation samples, *J.Chromatogr.A*, **1996**, *722*, 311–315.

SAMPLE

Matrix: food

Sample preparation: Homogenize (Sorvall) 5 g food with 50 mL 600 mM perchloric acid, centrifuge at 3500 rpm for 20 min, filter (0.45 μm) the supernatant, adjust the pH of the filtrate to 7.0 ± 0.2 with 30% KOH, place in the fridge for 5 min. Evaporate a 1 mL extract to dryness under reduced pressure at 37°, add 20 μL reagent, mix. Let stand at room temperature for 20 min, evaporate to dryness under reduced pressure at 37°, reconstitute with 200 μL buffer, inject a 20 μL aliquot. (Prepare reagent by mixing 70 μL EtOH, 10 μL water, 10 μL triethylamine, and 10 μL phenylisothiocyanate just before use. Buffer was MeCN:water containing 710 mg Na_2HPO_4, adjust pH to 7.40 with phosphoric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrabase C18

Mobile phase: Gradient. A MeCN:buffer 6:76. A was MeCN:water 60:40. A:B from 100:0 to 54:46 over 14.5 min, to 0:100 over 0.5 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 3.5 min. (Buffer was 19 g/L sodium acetate trihydrate containing 500 μL/L triethylamine, adjust pH to 6.40 with glacial acetic acid.)

Column temperature: 38 ± 1

Flow rate: 1 for 15 min, to 1.5 over 2 min, maintain at 1.5 for 3 min, return to 1 over 0.5 min

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4.2 (Asp), 5 (Glu), 8.2 (Ser), 8.5 (Gly), 9 (His), 9.7 (Arg), 10.2 (Thr), 10.8 (Ala), 12.3 (Pro), 13.5 (Tyr), 14.4 (Val), 15.1 (Met), 15.6 (Cys), 16.5 (Ile), 16.7 (Leu), 17.5 (Phe), 17.7 (Trp), 17.9 (Lys)

KEY WORDS
derivatization

REFERENCE
Alonso,M.L.; Alvarez,A.I.; Zapico,J. Rapid analysis of free amino acids in infant foods, *J.Liq.Chromatogr.*, **1994**, *17*, 4019–4030.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve formulations in 10-100 mM HCl, filter, analyze an aliquot. Peptides not containing Cys or Trp. Mix 500 μg peptide with 500 μL 6 M HCl, flush tube with nitrogen, heat at 110 ± 1° for 24 h, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL 100 mM HCl. Add a 2 μL aliquot to 5 μL 200 mM pH 10.4 borate buffer and 1 μL reagent, mix for 2 min, inject the whole amount. Peptides containing Trp. Hydrolyze 500 μg peptide with 500 μL 4 M methanesulfonic acid at 110° for 24 h, cool, add 500 μL water. Add a 2 μL aliquot to 5 μL 200 mM pH 10.4 borate buffer and 1 μL reagent, mix for 2 min, inject the whole amount. Peptides containing Cys. Dissolve 500 μg peptide in 1 mL formic acid, add 500 μL MeOH, cool to -10°, add 2.5 mL performic acid, let stand at -10° for 2.5 h, evaporate to dryness under reduced pressure, reconstitute with 500 μL 6 M HCl, flush tube with nitrogen, heat at 110 ± 1° for 24 h, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL 100 mM HCl. Add a 2 μL aliquot to 5 μL 200 mM pH 10.4 borate buffer and 1 μL reagent, mix for 2 min, inject the whole amount. (Prepare performic acid immediately before use by mixing 98% formic acid and 30% hydrogen peroxide in a 98:2 ratio, let stand for 2 h. Reagent was 260 mM N-isobutyryl-L-cysteine and 170 mM o-phthaldialdehydein 1 M potassium borate buffer (Pierce fluoraldehyde diluent).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil ODS
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. A was 23 mM pH 6.0 sodium acetate buffer. B was MeOH:MeCN 60:5. A:B from 100:0 to 46.5:53.5 over 75 min.
Column temperature: 25
Flow rate: 1
Injection volume: 8
Detector: F ex 230 em 445

CHROMATOGRAM
Retention time: 19 (L-Asp), 20 (D-Asp), 26 (L-Glu), 28 (D-Glu), 35 (L-Thr), 37 (Gly), 38 (L-His), 42 (L-Ala), 44 (L-Arg), 45 (D-Arg), 45.5 (D-Ala), 56 (L-Val), 57 (L-Met), 59 (L-Trp), 61 (D-Val), 62 (L-Phe), 63 (L-Ile), 65 (D-Phe), 67 (L-Leu), 70 (D-Leu), 72 (L-Lys)

KEY WORDS
capsules; tablets; pills; dragees; granulates; derivatization; chiral

REFERENCE
Brückner,H.; Westhauser,T.; Godel,H. Liquid chromatographic determination of D- and L-amino acids by derivatization with *o*-phthaldialdehyde and N-isobutyryl-L-cysteine. Applications with reference to the analysis of peptidic antibiotics, toxins, drugs and pharmaceutically used amino acids, *J.Chromatogr.A*, **1995**, *711*, 201–215.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate pills, capsules, powders, or drops in 50 mM sodium dodecyl sulfate, mix an aliquot with a 10-fold molar excess of reagent, let stand for 1 min, inject an aliquot. (Reagent was 2 mM o-phthalaldehyde and 2 mM N-acetyl-L-cysteine in 100 mM pH 9.5 sodium borate buffer.)

HPLC VARIABLES
Guard column: 35 × 4.6 5 μm Spherisorb ODS-2
Column: 120 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Propanol:50 mM sodium dodecyl sulfate 3:97, pH 3
Flow rate: 1
Injection volume: 20
Detector: UV 336

CHROMATOGRAM
Retention time: 2.5 (threonine), 3, 8 (lysine (different derivatives)), 3 (glycine), 6 (methionine)

KEY WORDS
pills; capsules; powders; drops; derivatization

REFERENCE
Catalá-Icardo,M.; Medina-Hernández,M.J.; García Alvarez-Coque,M.C. Determination of amino acids by micellar high-performance liquid chromatography and pre-column derivatization with o-phthalaldehyde and N-acetyl-L-cysteine, *J.Liq.Chromatogr.*, **1995**, *18*, 2827–2841.

SAMPLE
Matrix: fungal spore walls
Sample preparation: Hydrolyse fungal spore walls with 6 M HCl at 110° for 12 h, evaporate to dryness under reduced pressure, chromatograph on a DeltaPak C18 column (Waters) with 0.1% trifluoroacetic acid as mobile phase and detection at UV 214. Collect the unretained material and evaporate it to dryness under reduced pressure, dissolve 20 nmoles crude amino acids in 400 μL 100 mM pH 9 borate buffer, add 300 μL 10 mg/mL dansyl chloride in acetone, let stand in the dark for 2 h, evaporate to dryness under a stream of nitrogen, reconstitute with acetone:1 M HCl 95:5, centrifuge, evaporate the supernatant to dryness, repeat this extraction, reconstitute with mobile phase A, inject a 20 μL aliquot, elute to waste with mobile phase A, divert the fraction (200 μL) containing the amino acid from column A to column B (16.6-17 min for alanine; 24.5-24.8 min for glutamic acid), elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 250 × 4 7 μm LiChrosorb RP-18; B 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: A EtOH:25 mM pH 5.5 ammonium acetate containing 0.1% triethylamine 21:79 or 30:70; B EtOH:15 mM pH 5.5 ammonium acetate 20:80 containing 35 mM β-cyclodextrin and 1 M urea.
Flow rate: 0.5
Injection volume: 20
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 12.5 (D-Glu (mobile phase A 21:79)), 13.3 (L-Glu (mobile phase A 21:79)), 38.6 (D-Ala (mobile phase A 30:70)), 41.5 (L-Ala (mobile phase A 30:70))

KEY WORDS
derivatization; chiral; column-switching

REFERENCE
Rizzi,A.M.; Briza,P.; Breitenbach,M. Determination of D-alanine and D-glutamic acid in biological samples by coupled-column chromatography using β-cyclodextrin as mobile phase additive, *J.Chromatogr.*, **1992**, *582*, 35–40.

SAMPLE
Matrix: hair, protein

Sample preparation: Suspend 10 mg protein or hair in 10 mL 6 M HCl, seal tube under vacuum, heat at 110° for 20 h. Remove a 1 mL aliquot and evaporate it to dryness, dissolve the residue in 1 mL 100 mM HCl, make up to 10 mL with water, filter (Adovantex DISMIC-13cp), dilute the filtrate 10-fold with water. Place a 100 μL aliquot of 100 μg/mL solution of 1-methoxycarbonylindolizine-3,5-dicarbaldehyde in ethyl acetate in the bottom of a tube, evaporate to dryness under reduced pressure, add 100 μL reaction buffer, sonicate for 30 s, add 20 μL protein hydrolysate, mix well, let stand for 20 min in the dark, inject a 10 μL aliquot. (Prepare phosphate-borate buffer by mixing equal volumes of 20 mM NaH_2PO_4 and 20 mM sodium tetraborate, adjust pH to 10 with 1 M NaOH. Prepare reaction buffer by mixing equal volumes of EtOH and phosphate-borate buffer. Prepare reagent (1-methoxycarbonylindolizine-3,5-dicarbaldehyde) as follows. Reflux 21.4 g 2-pyridinecarboxaldehyde, 24 mL ethylene glycol, 10 g p-toluenesulfonic acid, and 300 mL benzene (Caution! Benzene is a carcinogen!) under a Dean-Stark separator for 64 h, pour into concentrated sodium carbonate solution. Remove the organic layer and extract the aqueous layer 4 times with benzene. Combine the organic layers and wash them with water, dry over anhydrous magnesium sulfate, evaporate, distil the residue to give 2-(1,3-dioxolan-2-yl)pyridine (bp 122°/4 mm Hg) (J.Org.Chem. 1963, 28, 83). Reflux 15.1 g 2-(1,3-dioxolan-2-yl)pyridine and 19.5 g tert-butyl bromoacetate in 100 mL dry acetonitrile for 7 h,let stand overnight at room temperature, filter, wash the precipitate with diethyl ether to give 1-(tert-butoxycarbonylmethyl)-2-(1,3-dioxolan-2-yl)pyridinium bromide (mp 110-2° from MeCN). Suspend 51.9 g of this compound in 1.5 L THF with stirring, add 62.1 g potassium carbonate, add 15.12 g methyl propiolate, stir at room temperature for 9 days, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with hexane:ethyl acetate 20:1-10:1, collect fractions and evaporate to dryness to give methyl 3-tert-butoxycarbonyl-5-(1,3-dioxolan-2-yl)indolizine-1-carboxylate (mp 138-9° from hexane). Reflux 20.82 g of this compound in 600 mL THF and 60 mL 10% HCl for 6 h, concentrate to one quarter of the original volume, add water, extract with chloroform. Wash the chloroform layer with water and dry it over anhydrous sodium sulfate, evaporate, chromatograph on silica gel with hexane:ethyl acetate 10:1 to give 1-methoxycarbonylindolizine-5-carbaldehyde (mp 135-7° from MeOH). Stir 12.18 g of this compound in 116 mL dry DMF at 0° under argon, add 17 mL phosphorus oxychloride, stir at room temperature for 1 h, pour into water, adjust pH to 9.0 with 5% potassium carbonate, extract with chloroform. Wash the organic layer with water and dry it over anhydrous sodium sulfate, concentrate until a precipitate forms, filter to obtain the product, concentrate the filtrate and chromatograph the residue on silica gel with hexane:ethyl acetate 5:1 to obtain more product. The product was 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (mp 164-5° from methyl acetate).)

HPLC VARIABLES

Column: 150 × 6 Asahipak ODP-50 (Asahi)

Mobile phase: Gradient. MeCN:buffer from 7:93 to 20:80 over 40 min, to 30:70 over 10 min, to 50:50 over 5 min, return to initial conditions over 5 min.) (Prepare buffer by adjusting the pH of 20 mM $(NH_4)H_2PO_4$ containing 10 mM sodium 1-octanesulfonate to 2.6 with phosphoric acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 10

Detector: F ex 414 em 482

CHROMATOGRAM

Retention time: 13.8 (Asp), 15.5 (Ser), 17 (Gly), 18.8 (Glu), 19.2 (Cys), 20 (Thr), 21.5 (Ala), 30 (His), 33.8 (Val), 34.6 (ammonia), 35.8 (Met), 38.3 (Arg), 39.2 (Lys), 42.5 (Ile), 44.2 (Leu), 46.7 (Phe)

Limit of detection: 0.2-200 fmole

KEY WORDS

derivatization; soybean

REFERENCE

Oguri,S.; Uchida,C.; Mishina,M.; Miki,Y.; Kakehi,K. Determination of amino acids by pre-column fluorescence derivatization with 1-methoxycarbonylindolizine-3,5-dicarbaldehyde, *J.Chromatogr.A*, **1996**, *724*, 169–177.

SAMPLE

Matrix: peptides

Sample preparation: Freeze-dry 0.1-1 μg peptide containing 1-10 nmole total amino acids, add 20 μL 6 M HCl, seal in a tube under reduced pressure, heat at 110° for 24 h, evaporate under vacuum, add 10 μL 100 mM pH 9.0 sodium bicarbonate buffer, add 20 μL 4 mM dimethylaminoazobenzenesulfonyl chloride (dabsyl chloride) in acetone, heat at 70° for 10-15 min with occasional shaking, dilute to 100-500 μL with EtOH:water 70:30, inject a 10 μL aliquot. (Recrystallize dimethylaminoazobenzenesulfonyl chloride from acetone.)

HPLC VARIABLES

Column: Zorbax ODS

Mobile phase: Gradient. G1 = MeCN:buffer from 20:80 to 70:30 over 25 min, stay at 70:30 for 5 min, wash with 100:0 for 15 min, return to initial conditions over 5 min, re-equilibrate for 10 min. (Buffer was 5.44 g sodium acetate trihydrate and 7.7 mL acetic acid made up to 900 mL with water, pH 4.13.) (Using G2 = MeCN:pH 7.2 phosphate buffer from 20:80 to 35:65 over 15 min, stay at 35:65 for 5 min, go to 65:35 over 5 min, stay at 65:35 for 5 min, Asp and Ser are separated but other separations are not as good.)

Flow rate: 1.2

Injection volume: 10

Detector: UV 436

CHROMATOGRAM

Retention time: 13.5 (8.5) (Asp) (for G1, G2 times in parentheses), 13.5 (14.5) (Ser), 14 (9) (Glu), 14.5 (15) (Thr), 15 (15.5) (Gly), 16 (18) (Arg), 17 (16) (Ala), 19 (18.5) (Met), 20 (17) (Pro), 20.5 (17.5) (Val), 21 (21) (Phe), 22.5 (19.2) (Leu), 23 (18.8) (Ile), 26 (27.3) (His), 27 (27) (Lys), 30 (28) (Tyr)

KEY WORDS

derivatization; dabsylation; dabsyl; Asp and Ser co-elute

REFERENCE

Chang,J.-Y.; Knecht,R.; Braun,D.G. Amino acid analysis at the picomole level. Application to the C-terminal sequence analysis of polypeptides, *Biochem.J.*, **1981**, *199*, 547–555.

SAMPLE

Matrix: peptides

Sample preparation: Freeze dry 0.1-1 μg peptide in a tube, add 20 μL 6 M HCl, seal under reduced pressure, heat at 120° for 24 h, centrifuge, evaporate to dryness, reconstitute with 10 μL 200 mM pH 9.0 sodium bicarbonate buffer, add 20 μL 4 mM dabsyl chloride in acetone, heat at 70° with occasional shaking for 10-15 min, dilute to 100-500 μL with EtOH:water 70:30 or MeCN:water 50:50, inject a 10 μL aliquot.

HPLC VARIABLES

Column: Lichrosorb C-18

Mobile phase: Gradient. A was DMF:17 mM pH 6.5 phosphate buffer 2:98. B was MeCN:DMF 96:4. A:B from 85:15 to 55:45 over 25 min, to 30:70 over 10 min.

Column temperature: 50

Flow rate: 1

Injection volume: 10

Detector: UV 436

CHROMATOGRAM

Retention time: 11 (Asp), 12 (Glu), 18 (Ser), 18.7 (Thr), 19.3 (Gly), 20 (Ala), 21.5 (Pro), 21.7 (Val), 22.2 (Arg), 23 (Met), 24 (Ile), 24.5 (Leu), 25 (Phe), 32.5 (ammonia), 34.5 (Lysine), 35 (His), 36.5 (Tyr)

KEY WORDS

derivatization

REFERENCE

Chang,J.-Y.; Knecht,R.; Braun,D.G. Amino acid analysis in the picomole range by precolumn derivatization and high-performance liquid chromatography, *Methods Enzymol.*, **1983**, *91*, 41–48.

SAMPLE

Matrix: peptides

Sample preparation: Heat 0.5-5 mg peptide with 1 mL 1% indolylpropionic acid in'6 M HCl under vacuum in a sealed tube at 115° for 4 h, evaporate to dryness, reconstitute with 10-100 mL water, remove a 1 mL aliquot, add 100 μL 20 mM dithioerythritol, add 100 μL 400 mM iodomethane in MeOH:water 50:50, add 200 μL 3 M NaOH, let stand for 10 min, add 200 μL 3 M HCl, mix briefly, add 400 μL 400 mM pH 10 sodium borate buffer, mix, check that pH is about 10. Remove a 100 μL aliquot, add 400 μL reagent, mix, let stand for 10 min, inject a 25 μL aliquot. (Iodomethane is used to protect cysteine as its S-methyl derivative. Prepare reagent by dissolving 30 mg o-phthaldialdehyde in 1 mL EtOH, add 22 mL 400 mM pH 10 sodium borate buffer, add 30 mg Boc-L-cysteine. Prepare Boc-L-cysteine by adding Boc-S-benzyl-L-cysteine to liquid ammonia, add metallic sodium until a blue color persists for 15 min, add ammonium chloride until all the excess sodium is destroyed, allow the ammonia to evaporate, add ice and water, extract with ethyl acetate.)

HPLC VARIABLES

Column: 120 × 4 3 μm Hypersil ODS

Mobile phase: Gradient. A was 50 mM pH 7.0 phosphate buffer. B was MeOH:THF:50 mM pH 7.0 phosphate buffer 65:1:35. A:B from 70:30 to 0:100 over 48 min.

Flow rate: 1.4

Injection volume: 25

Detector: F ex 344 em 443

CHROMATOGRAM

Retention time: 20.5 (L-Thr), 22.5 (D-Thr), 28.5 (S-methyl-L-Cys), 31 (L-threoninol), 31.5 (S-methyl-D-Cys), 32 (D-threoninol), 34 (L-Trp), 36.5 (L-Phe), 37 (D-Trp), 38 (D-Phe), 47 (L-Lys), 48 (D-Lys)

KEY WORDS

derivatization; chiral; comparison with the results obtained with other thiols in the derivatization reagent

REFERENCE

Buck,R.H.; Krummen,K. High-performance liquid chromatographic determination of enantiomeric amino acids and amino alcohols after derivatization with *o*-phthaldialdehyde and various chiral mercaptans. Application to peptide hydrolysates, *J.Chromatogr.*, **1987**, *387*, 255–265.

SAMPLE

Matrix: peptides

Sample preparation: Mix ≤5 mg peptide and 200 μL 6 M HCl, heat at 100° for 6 h, evaporate to dryness under a stream of nitrogen at 80-100°, reconstitute with 3 M HCl in isopropanol, heat at 105° for 20 min, evaporate to dryness under a stream of nitrogen at 105°, reconstitute with 1 mL 5 mg/mL sodium heptanesulfonate in water, extract with 5 mL dichloromethane. Evaporate the organic layer to dryness under a stream of nitrogen

at 50°, add 20 mg anhydrous sodium carbonate, add a 3-fold molar excess of 1 mg/mL S-flunoxaprofen chloride in dichloromethane or ethyl acetate, heat at 40° for 1 h (or agitate moderately overnight), evaporate to dryness, reconstitute with 200 μL mobile phase, inject a 10-20 μL aliquot. (Prepare S-flunoxaprofen chloride as follows. Dissolve 1 mmole S-flunoxaprofen in 25 mL toluene, add a trace of DMF (this paper), add 2.5 mL thionyl chloride, reflux for 30 min, remove solvent by evaporation, dry the residue under vacuum over KOH, recrystallize from dichloromethane (mp 73°) (J.Chromatogr. 1988, 427, 131).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax-Sil
Mobile phase: n-Hexane:chloroform:EtOH 100:10:1
Flow rate: 2
Injection volume: 10-20
Detector: UV 305 or F ex 305 em 355

CHROMATOGRAM

Retention time: k' 11.2 (D-alanine), k' 15.2 (L-alanine), k' 19.5 (glycine), k' 4.8 (D-isoleucine), k' 4.5 (L-isoleucine), k' 7.1 (D-leucine), k' 6.0 (L-leucine), k' 11.9 (D-methionine), k' 10.5 (L-methionine), k' 6.6 (D-phenylalanine), k' 6.4 (L-phenylalanine), k' 15.0 (D-proline), k' 12.3 (L-proline), k' 22.6 (D-tyrosine), k' 13.1 (L-tyrosine)
Limit of detection: 0.1-0.5 ng

KEY WORDS

normal phase; derivatization; chiral

REFERENCE

Langguth,P.; Spahn,H.; Merkle,H.P. Fluorescence assay for small peptides and amino acids: high-performance liquid chromatographic determination of selected substrates using activated *S*-flunoxaprofen as a chiral derivatizing agent, *J.Chromatogr.*, **1990**, *528*, 55–64.

SAMPLE

Matrix: peptides
Sample preparation: Dry 0.5-1 nmole peptide in a tube. add 500 μL 6 M HCl, evacuate, flush with nitrogen, evacuate, seal, heat in the vapor phase at 110° for 20-24 h, evaporate to dryness under reduced pressure, reconstitute with 5 μL 200 mM sodium bicarbonate, dry under reduced pressure, add 5 μL water, add a 2- to 3-fold molar excess of reagent in 10 μL acetone, heat in the dark with gentle shaking at 50° for 1 h, evaporate to dryness under reduced pressure, reconstitute, inject an aliquot. (Synthesize the reagent, 2,4-dinitrophenyl-1-fluoro-5-L-alanine, as follows. Add 10 mL 200 mM 1,5-difluoro-2,4-dinitrobenzene in acetone dropwise with stirring to 10 mM 200 mM L-alanine in 5 mM sodium bicarbonate, stir in the dark at room temperature at pH 8 for 4 h, acidify to pH 3 with 2 M HCl, evaporate to dryness, take up in 100 mM sodium bicarbonate, extract with diethyl ether, crystallize by the addition of hot 2 M HCl, filter, wash with acidified water, dry in the air in the dark to give 2,4-dinitrophenyl-1-fluoro-5-L-alanine as golden-yellow scales (mp 136-137°).)

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl
Mobile phase: Gradient. A was 40 mM pH 2.2 triethylammonium phosphate. B was MeCN: isopropanol 80:20. A:B from 76:24 to 61:39 over 38 min, to 35:65 over 32 min, to 5:95 over 0.2 min, maintain at 5:95 for 3.8 min, return to initial conditions over 0.2 min.
Column temperature: 25
Flow rate: 0.2
Injection volume: 20
Detector: UV 340

CHROMATOGRAM

Retention time: 7 (D-His), 8 (L-His), 9.5 (D-Arg), 10 (L-Arg), 17 (L-Ser), 18 (D-Ser), 19 (L-Thr), 20.5 (L-Asp), 21.5 (D-Asp), 23.5 (L-Glu), 24.5 (D-Glu), 25 (D-Thr), 25.5 (Gly), 29.5 (ammonia), 30 (L-Ala), 37 (D-Ala), 40.5 (L-Met), 43.5 (L-Val), 44.5 (L-norvaline), 49 (D-Met), 50.5 (L-Phe), 51 (L-Ile), 51.5 (L-Leu), 52 (L-Lys, D-Val), 55 (D-Lys), 56 (D-Phe), 57.5 (D-Ile), 58.5 (D-Leu), 60 (L-Tyr), 65 (D-Tyr)

KEY WORDS

derivatization; chiral

REFERENCE

Scaloni,A.; Simmaco,M.; Bossa,F. Determination of the chirality of amino acid residues in the course of subtractive Edman degradation of peptides, *Anal.Biochem.*, **1991**, *197*, 305–310.

SAMPLE

Matrix: peptides

Sample preparation: Heat 60 nmole peptide with 100 μL acetic anhydride at 50° for 10 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL water:triethylamine 98:2, let stand at 25° for 5 min, evaporate to dryness under reduced pressure, reconstitute with 40 μL anhydrous MeCN, add 10 μL pyridine, add 60 μL 1 mM diphenyl phosphoroisothiocyanate in MeCN, heat at 50° for 30 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL 0.1% trifluoroacetic acid in water, inject an aliquot. (Synthesis of diphenyl phosphoroisothiocyanate is as follows. Add 50.1 g diphenyl chlorophosphate to a solution of 19.3 g potassium thiocyanate in 200 mL MeCN, shake for 3 h, let stand for 3 h, dilute with 300 mL dry benzene (Caution! Benzene is a carcinogen!), filter, evaporate the filtrate, distil the residue to give diphenyl phosphoroisothiocyanate (bp 105°/0.1 mm Hg) (J. Chem. Soc. 1953, 673).)

HPLC VARIABLES

Column: 250 × 2.0 3 μm Reliasil C18 (Column Engineering, Ontario CA)

Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was MeCN:MeOH: water 80:10:10. A:B 100:0 for 2 min, to 96:4 over 3 min, to 65:35 over 35 min, to 50:50 over 10 min, return to initial conditions over 2 min.

Detector: UV 265

CHROMATOGRAM

Retention time: 7 (asparagine), 8 (glycine), 10 (aspartic acid), 12.5 (glutamine), 13.5 (histidine), 14 (alanine), 15.5 (glutamic acid), 17 (serine), 19 (arginine), 21 (cysteine), 23 (lysine), 23.5 (threonine), 26 (valine), 28 (tyrosine), 28.5 (methionine), 35.5 (isoleucine), 38 (leucine), 41 (phenylalanine), 44 (tryptophan)

KEY WORDS

derivatization

REFERENCE

Bailey,J.M.; Nikfarjam,F.; Shenoy,N.R.; Shively,J.E. Automated carboxy-terminal sequence analysis of peptides and proteins using diphenyl phosphoroisothiocyanatidate, *Protein Sci.*, **1992**, *1*, 1622–1633.

SAMPLE

Matrix: peptides

Sample preparation: Dry a solution containing 1 μg peptide under vacuum, add 200 μL 0.06% phenol in constant-boiling HCl, evacuate and purge with nitrogen 3 times, evacuate and seal, heat at 114° for 22 h, cool, evaporate to dryness under reduced pressure, reconstitute with 20 μL 20 mM HCl, add 60 μL 200 mM pH 8.8 sodium borate containing 5 mM EDTA, add 20 μL 3 mg/mL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) in MeCN, inject an aliquot. (6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate can be purchased from Waters or synthesized as follows. Reflux 3 g N,N'-succinimidyl carbonate in

100 mL dry MeCN, add 1.5 g 6-aminoquinoline in 50 mL dry MeCN dropwise over 30 min, reflux for 30 min, evaporate to half volume under reduced pressure, cool for 24 h, filter, wash the solid with cold MeCN, recrystallize from MeCN to obtain 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate as off-white crystals (mp 210-215° (d)) (Anal. Biochem. 1993, 211, 279).)

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18

Mobile phase: Gradient. MeCN:buffer:water 0:100:0 for 0.5 min, to 1:99:0 (step gradient), to 5:95:0 over 17.5 min, to 9:91:0 over 1 min, to 17:83:0 over 10.5 min, to 32:68:0 over 3.5 min, to 60:0:40 (step gradient), maintain at 60:0:40 for 3 min, re-equilibrate at initial conditions for 9 min. (Buffer was 140 mM sodium acetate containing 17 mM triethylamine and 3 mM EDTA, adjusted to pH 5.05 with phosphoric acid.)

Column temperature: 37

Flow rate: 1

Detector: F ex 250 em 395

CHROMATOGRAM

Retention time: 14 (Asp), 15 (Ser), 16 (Glu), 17 (Gly), 17.5 (His), 19 (ammonia), 21.8 (Arg), 22 (Thr), 23 (Ala), 24 (Pro), 26.8 (Cys), 27 (Tyr), 28.3 (Val), 28.7 (Met), 30.7 (Lys), 31.8 (Ile), 32.5 (Leu), 33.5 (Phe)

Limit of detection: 38-794 fmole

KEY WORDS

derivatization; procedure can also be used to derivatize peptides; changes to the gradient are required.

REFERENCE

De Antonis,K.M.; Brown,P.R.; Cohen,S.A. High-performance liquid chromatographic analysis of synthetic peptides using derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, *Anal.Biochem.*, **1994**, *223*, 191–197.

SAMPLE

Matrix: peptides

Sample preparation: Lyophilize 100 μL peptide solution, add 100 μL 6 M HCl, heat at 105° in a sealed glass tube for 16 h, lyophilize 50 μL of the hydrolyzate, reconstitute with 15 μL 200 mM pH 8.5 sodium bicarbonate solution, add 15 μL 0.015% acenaphthene-5-sulfonyl chloride in dry acetone, mix, heat at 47° for 30 min, cool, add 100 μL 2% ethylamine, inject a 20 μL aliquot. (Preparation of acenaphthene-5-sulfonyl chloride is as follows. Dissolve 20 g acenaphthene in 100 g nitrobenzene, cool to 0°, add 9 mL chlorosulfonic acid dropwise with stirring, maintain the temperature below 5°, when the addition is complete allow the temperature to rise to 20° over 30 min, add 500 mL water. Remove the aqueous layer and neutralize it with solid sodium carbonate, heat and add NaCl until precipitation occurs, cool in an ice bath for 1 h, filter, heat at 140° to remove traces of water and nitrobenzene to give acenaphthene-5-sulfonic acid sodium salt as a pale yellow solid (mp >300°). Grind 10 g acenaphthene-5-sulfonic acid sodium salt with 3.5 g phosphorus pentachloride in a mortar for 3 min, add ice and water, extract with 100 mL ethyl acetate. Wash the ethyl acetate layer with 5% sodium bicarbonate and with water until neutral, dry over anhydrous sodium sulfate, evaporate the ethyl acetate under a stream of nitrogen, chromatograph on a 300 × 20 column of silica gel H with toluene to give acenaphthene-5-sulfonyl chloride (mp 98-101°) as the first yellow band to elute.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil C18

Mobile phase: Gradient. A was THF:10 mM pH 4.2 acetate buffer 5:95. B was THF:MeCN 10:90. A:B from 90:10 to 60:40 over 30 min, maintain at 60:40 for 15 min, 0:100 for 3 min.

Flow rate: 1

Injection volume: 20
Detector: F ex 230 em 420

CHROMATOGRAM

Retention time: 7.5 (asparagine), 8.5 (glycine), 9.5 (serine), 10 (glutamine), 11 (alanine), 12.5 (arginine), 18.5 (ethylamine), 22.5 (valine), 26.5 (proline), 28 (tryptophan), 32.5 (lysine), 40 (tyrosine)

KEY WORDS

derivatization

REFERENCE

Gifford,L.A.; Owusu-Daaku,F.T.K.; Stevens,A.J. Acenaphthene fluorescence derivatization reagents for use in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *715*, 201–212.

SAMPLE

Matrix: peptides
Sample preparation: Mix 10 μL of a solution containing 50 pmole peptide with 35 μL reagent, heat at 50° for 10 min, evaporate to dryness at 50° in a centrifugal evaporator for 5 min, reconstitute with 10 μL water, wash with three 100 μL portions of n-heptane: dichloromethane 90:10, evaporate to dryness at 50° in a centrifugal evaporator for 15 min, reconstitute with 30 μL trifluoroacetic acid, heat at 50° for 5 min, evaporate to dryness under a stream of nitrogen. Reconstitute with 20 μL water, add 100 μL n-heptane:dichloromethane 70:30, mix, centrifuge at 1000 g for 5 min, repeat extraction 3 times. (The aqueous phase contains the residual peptide and can be subjected to the same procedure to determine the next amino acid.) Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 20 μL water:trifluoroacetic acid 80: 20, heat at 50° for 10 min, dry under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot. (Reagent was phenylisothiocyanate:EtOH:pyridine 1:4:2.)

HPLC VARIABLES

Column: 150 × 6 5 μm ES-1/2phCD (β-cyclodextrin 50% modified with phenylcarbamoyl) (Shinwakakou, Kyoto) (Column A) or 150 × 6 5 μm Ultron ES-phCD (phenylcarbamoylated β-cyclodextrin) (Shinwakakou, Kyoto) (Column B)
Mobile phase: MeOH:water 25:75 containing 10 mM formic acid (Mobile Phase A) or MeCN:MeOH:water 10:45:45 containing 10 mM formic acid (Mobile Phase B)
Flow rate: 0.7
Detector: UV 269

CHROMATOGRAM

Retention time: 15 (Gly) (Column A, Mobile Phase A), 16 (L-Ala) (Column A, Mobile Phase A), 17 (D-Ala) (Column A, Mobile Phase A), 17 (D-Leu) (Column B, Mobile Phase B), 17.5 (L-Leu) (Column B, Mobile Phase B), 21 (D-Phe) (Column B, Mobile Phase B), 22.5 (L-Tyr) (Column A, Mobile Phase A), 23 (L-Phe) (Column B, Mobile Phase B), 24 (D-Tyr) (Column A, Mobile Phase A)

KEY WORDS

chiral; derivatization

REFERENCE

Imai,K.; Matsunaga,H.; Santa,T.; Homma,H. Availability of phenylisothiocyanate for the amino acid sequence/configuration determination of peptides containing D/L-amino acids, *Biomed.Chromatogr.*, **1995**, *9*, 195–196.

SAMPLE

Matrix: peptides
Sample preparation: Vortex 10 μL of a 50-100 μM solution of a dipeptide in pyridine: water 50:50 with 10 μL 20 mM 7-N,N-dimethylaminosulfonyl-4-(2,1,3-benzoxadiazolyl)

isothiocyanate in pyridine:water 50:50, heat at 50° for 15 min, wash 3 times with 100 μL portions of n-heptane:dichloromethane 80:20. Evaporate the aqueous layer to dryness at 50° for 15 min, add 30 μL 1% boron trifluoride in dichloromethane containing 0.02% ethanethiol, heat at 50° for 5 min, evaporate to dryness under a stream of nitrogen, add 20 μL water, add 100 μL n-heptane:dichloromethane 70:30, mix, centrifuge at 1000 g for 5 min, repeat the extraction 3 times. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in MeCN, inject an aliquot. (Synthesis of 7-N,N-dimethylaminosulfonyl-4-(2,1,3-benzoxadiazolyl) isothiocyanate is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 μL 28% ammonia in water to 50 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 15 mL MeCN, stir at room temperature overnight, filter, evaporate the filtrate to dryness under reduced pressure, recrystallize from MeCN to give 4-amino-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as pale yellow needles (mp 214-217°). Add 1 mL 30% thiophosgene in benzene dropwise to 200 mg 4-amino-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 15 mL MeCN, reflux for 5 h, concentrate under reduced pressure, extract the residue twice with 20 mL portions of chloroform. Combine the chloroform layers, filter, evaporate the filtrate to dryness, chromatograph the residue on 15 g silica gel G-200 with chloroform. Collect the fraction containing the product and evaporate it to dryness under reduced pressure, recrystallize from benzene/n-hexane to obtain 7-N,N-dimethylaminosulfonyl-4-(2,1,3- benzoxadiazolyl) isothiocyanate as pale yellow-white crystals (mp 122-124°; yield 26%) (Biomed. Chromatogr. 1993, 7, 56).)

HPLC VARIABLES
Column: 150 × 6 5 μm ES-1/4phCD phenylcarbamoylated β-cyclodextrin (Shinwa, Kyoto)
Mobile phase: MeCN:MeOH:water 15:40:45 containing 10 mM acetic acid
Injection volume: 20

Detector: F ex 387 em 524

CHROMATOGRAM

Retention time: 21 (D-Pro), 23 (L-Pro), 27 (L-Leu), 30 (D-Leu)

KEY WORDS

derivatization; chiral

REFERENCE

Matsunaga,H.; Iida,T.; Fukushima,T.; Santa,.; Homma,H.; Imai,K. Boron-trifluoride etherate (Lewis acid) as an efficient acid at cyclization/cleavage reaction of D/L-amino acids affording the retention of their original configuration in the Edman sequencing method of peptides, *Biomed.Chromatogr.*, **1996**, *10*, 95–96.

SAMPLE

Matrix: peptides

Sample preparation: Mix 10 μL of a 50 μM peptide solution in pyridine:water 50:50 with 10 μL 20 mM 7-N,N-dimethylaminosulfonyl-4-(2,1,3-benzoxadiazolyl) isothiocyanate in pyridine:water 50:50, vortex, heat at 50° for 15 min, wash three times with 100 μL portions of n-heptane:dichloromethane 80:20, dry at 50° under reduced pressure for 15 min, add 30 μL 1% boron trifluoride etherate in dichloroethane containing 0.1% ethanethiol, heat at 50° for 5 min, evaporate to dryness under a stream of nitrogen, add 20 μL water, add 100 μL ethyl acetate:benzene 75:25 (Caution! Benzene is a carcinogen!), mix, centrifuge at 1000 g for 1 min, repeat the extraction 3 times. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. (Evaporate the aqueous phase to dryness and continue the cycle. Boron trifluoride gives less racemization than trifluoroacetic acid. Synthesis of 7-N,N-dimethylaminosulfonyl-4-(2,1,3-benzoxadiazolyl) isothiocyanate is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylami-

nosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 μL 28% ammonia in water to 50 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 15 mL MeCN, stir at room temperature overnight, filter, evaporate the filtrate to dryness under reduced pressure, recrystallize from MeCN to give 4-amino-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as pale yellow needles (mp 214-217°). Add 1 mL 30% thiophosgene in benzene dropwise to 200 mg 4-amino-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 15 mL MeCN, reflux for 5 h, concentrate under reduced pressure, extract the residue twice with 20 mL portions of chloroform. Combine the chloroform layers, filter, evaporate the filtrate to dryness, chromatograph the residue on 15 g silica gel G-200 with chloroform. Collect the fraction containing the product and evaporate it to dryness under reduced pressure, recrystallize from benzene/n-hexane to obtain 7-N,N-dimethylaminosulfonyl-4-(2,1,3-benzoxadiazolyl) isothiocyanate as pale yellow-white crystals (mp 122-124°; yield 26%) (Biomed. Chromatogr. 1993, 7, 56).)

HPLC VARIABLES

Column: 150 × 6 5 μm ES-1/4phCD 25% phenylcarbamoylated β-cyclodextrin (Shinwa)
Mobile phase: MeOH:water 70:30 containing 10 mM acetic acid
Column temperature: 5
Flow rate: 0.5
Injection volume: 20
Detector: F ex 387 em 524

CHROMATOGRAM

Retention time: 19 (L-Arg), 21 (D-Arg), 30 (L-Phe), 33 (D-Phe), 35 (L-Met), 38 (D-Met)

KEY WORDS

derivatization; chiral

REFERENCE

Matsunaga,H.; Santa,T.; Iida,T.; Fukushima,T.; Homma,H.; Imai,K. Proton: A major factor for the racemization and the dehydration at the cyclization/cleavage stage in the Edman sequencing method, *Anal.Chem.*, **1996**, *68*, 2850–2856.

SAMPLE

Matrix: perfusate
Sample preparation: Dry 120 μL perfusate under vacuum, add 50 μL EtOH:water:triethylamine 40:40:20, vortex, dry under vacuum, add 20 μL EtOH:water:triethylamine: phenylisothiocyanate 70:10:10:10, vortex, let stand at room temperature for 20 min, vacuum dry for at least 8 h (most of the derivatizing reagent must be removed in the first hour), reconstitute with 100 μL mobile phase A, inject a 70 μL aliquot.

HPLC VARIABLES

Guard column: 30-40 μm pellicular RP18
Column: 150 × 3.9 Novapak C18
Mobile phase: Gradient. A was MeCN:14 mM sodium acetate:triethylamine 4.5:95.5:0.05, adjusted to pH 6.6 with glacial acetic acid. B was MeCN:water 60:40. A:B 100:0 for 1 min, to 95:5 (step gradient), maintain at 95:5 for 9 min, to 94:6 (step gradient), to 0:100 over 4 min, maintain at 0:100 for 4 min, return to initial conditions, re-equilibrate for 10 min.
Column temperature: 37
Flow rate: 1.1 for 10 min, to 1.5 over 4 min, maintain at 1.5
Injection volume: 70
Detector: UV 254

CHROMATOGRAM
Retention time: 2 (aspartate), 2.4 (glutamate), 3.1 (adenosine), 3.4 (hydroxyproline), 5 (glutamine), 6.4 (taurine), 6.7 (gamma-aminobutyric acid), 9 (kainic acid)

KEY WORDS
derivatization

REFERENCE
Rogers,K.L.; Philibert,R.A.; Allen,A.J.; Molitor,J.; Wilson,E.J.; Dutton,G.R. HPLC analysis of putative amino acid neurotransmitters released from primary cerebellar cultures, *J.Neurosci.Methods*, **1987**, *22*, 173–179.

SAMPLE
Matrix: perfusate
Sample preparation: Lyophilize perfusate, reconstitute with water. Remove a 400 μL aliquot and add it to 100 μL 100 mM pH 6.2 borate buffer, adjust pH to 7.7 with 100 mM NaOH, add 500 μL 15.4 mM N-(9-fluorenylmethoxycarbonyloxy)succinimide (9-fluorenylmethyl N-succinimidyl carbonate) in acetone, let stand at room temperature for 1 min, extract twice with 2 mL portions of pentane. Evaporate the aqueous layer to dryness under reduced pressure, reconstitute with 200 μL water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: Gradient. MeCN:35 mM pH 5.5 ammonium acetate from 20:80 to 25:75 over 10 min, maintain at 25:75 for 5 min, to 50:50 over 25 min, to 80:20 over 10 min.
Flow rate: 1
Injection volume: 10
Detector: F ex 254 em 313

CHROMATOGRAM
Retention time: 14.5 (Asp), 15 (Glu), 20 (Asn), 21 (Gln), 22.5 (Ser), 26.5 (Thr, Gly), 27.5 (Tau), 29 (Ala), 32.5 (Arg, Pro), 33 (β-Ala), 36 (Met), 37 (Bal), 39 (Phe), 39.5 (Ile, Leu)

KEY WORDS
derivatization

REFERENCE
Keller,H.J.; Do,K.Q.; Zollinger,M.; Winterhalter,K.H.; Cuénod,M. Cysteine: depolarization-induced release from rat brain in vitro, *J.Neurochem.*, **1989**, *52*, 1801–1806.

SAMPLE
Matrix: perfusate
Sample preparation: Add L-norvaline to a concentration of 750 μM, remove a 1 mL aliquot and add it to 75 μL ice-cold 60% perchloric acid, centrifuge, neutralize supernatant with 200 mM potassium carbonate, add (?) 0.5 M potassium carbonate:potassium hydrogen carbonate 30:70, adjust pH to 9.40-9.50 with 5 M KOH. Remove a 200 μL aliquot and add it to 100 μL 1.25 mg/mL dansyl chloride in MeCN, let stand at room temperature in the dark for 1 h, add 6 μL 0.2% triethylamine in water, add acetic acid to a final concentration of 3%, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Biosil ODS-5S microguard refill cartridge (Biorad)
Column: 250 × 4 Biosil C18 ODS-5S (Biorad)
Mobile phase: Gradient. A was MeOH:water 15:85 containing 1% glacial acetic acid and 0.030% triethylamine. B was MeOH:MeCN 70:30 containing 3% glacial acetic acid and

0.030% triethylamine. A:B from 70:30 to 50:50 over 52 min, to 25:75 over 21 min, maintain at 25:75 for 5 min, reset to initial conditions over 7 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 520

CHROMATOGRAM
Retention time: 10 (Asn), 11 (Gln), 13 (Ser), 14 (Glu), 15 (Asp), 17 (Gly), 18 (Thr), 22 (Ala), 24 (Arg), 32 (Pro), 35 (Met), 37 (Val), 45 (Try), 53 (Phe), 55 (Iso-leu), 57 (Leu), 73 (Orn), 75 (Lys), 79 (His), 83 (Tyr)
Internal standard: L-norvaline (41)

KEY WORDS
rat; derivatization; dansylation

REFERENCE
Zezza,F.; Kerner,J.; Pascale,M.R.; Giannini,R.; Arrigoni Martelli,E. Rapid determination of amino acids by high-performance liquid chromatography: release of amino acids by perfused rat liver, *J.Chromatogr.*, **1992**, *593*, 99–101.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeCN:MeOH:139 mM Na_2HPO_4 3.1:25:71.9 adjusted to pH 6.8 with phosphoric acid
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 450 mV

CHROMATOGRAM
Retention time: 5.56 (Asp), 6.28 (Glu), 7.50 (Asn), 8.48 (His), 8.76 (Ser), 9.22 (Gln), 10.70 (Arg), 12.75 (Gly), 13.40 (Thr), 18.99 (Tau), 21.69 (Ala), 24.12 (GABA), 25.02 (Tyr)
Limit of detection: 0.75 ng/mL

KEY WORDS
rat; pharmacokinetics

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: protein
Sample preparation: Hydrolyze 500 ng protein with 400 μL 6 M HCl at 110° for 24 h (flush tube with argon and evacuate to 0.1 mbar before sealing, alternatively hydrolyze

in liquid phase with 25 μL 6 M HCl), evaporate to dryness under reduced pressure, reconstitute with 20 μL 50 mM pH 8.1 sodium bicarbonate, add 40 μL 4 mM dabsyl chloride in MeCN, heat at 70° for 10 min, dilute to 1 mL with EtOH:50 mM pH 7.0 sodium phosphate 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 5 μm Lichrosphere 100 CH-18/2
Mobile phase: Gradient. A was DMF:25 mM sodium acetate 4:96, pH 2.5. B was MeCN. A:B from 85:15 to 60:40 over 20 min, to 30:70 over 12 min, maintain at 30:70 for 2 min, return to initial conditions over 2 min, re-equilibrate for 8 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 436

CHROMATOGRAM

Retention time: 15 (Aspartate), 16 (Glutamate), 20.5 (Serine), 21 (Threonine), 22 (Glycine), 22.5 (Alanine), 23 (Arginine), 23.5 (Proline), 24 (Valine), 25 (Methionine), 20.5 (Isoleucine), 21 (Leucine), 22 (Phenylalanine), 23.5 (Cysteine), 32 (ammonia), 33.5 (Lysine), 34 (Histidine), 35.5 (Tyrosine)
Limit of detection: <0.5 pmole

KEY WORDS

derivatization

REFERENCE

Knecht,R.; Chang,J.Y. Liquid chromatographic determination of amino acids after gas-phase hydrolysis and derivatization with (dimethylamino)azobenzenesulfonyl chloride, *Anal.Chem.*, **1986**, *58*, 2375–2379.

SAMPLE

Matrix: protein
Sample preparation: Heat 5 mg albumin with 1 mL 6 M HCl, 10 μL mercaptoethanol and 10 μL octanol under vacuum at 110° for 24 h, evaporate to dryness, take up in 1 M HCl, add to a 100 × 5 Dowex 50 ion-exchange column, elute with 1 M HCl, collect fractions. Evaporate fractions to dryness, reconstitute with 2 mL 3% sodium bicarbonate solution, add 4 mL 25 μL/mL fluorodinitrobenzene, let stand in the dark at room temperature overnight, wash with ether, add 2 drops 6 M HCl, extract with ether. Evaporate the ether layer to dryness, reconstitute with EtOH, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeOH:30 mM pH 5 KH_2PO_4 from 40:60 to 55:45 over 30 min, maintain at 55:45 for 5 min, return to initial conditions over 5 min, re-equilibrate for 5 min.
Flow rate: 1
Detector: UV 365

CHROMATOGRAM

Retention time: 5 (proline), 10 (tyrosine), 11 (s-methylcysteine), 18 (methionine), 20 (valine), 21 (cystine), 29 (phenylalanine), 31 (isoleucine, leucine)

KEY WORDS

derivatization

REFERENCE

Seago,A.; Shuker,D.E.G.; Paine,A.J. Interaction of [^{14}C]dimethylnitrosamine with albumin produced by rat hepatocytes in culture, *Toxicol.Lett.*, **1986**, *30*, 41–48.

SAMPLE

Matrix: protein

Sample preparation: Grind soybeans to a fine powder, add 6 M HCl, heat at 110° for 22 h, evaporate to dryness in a desiccator over NaOH pellets, reconstitute with EtOH:0.2 N pH 3.2 sodium citrate 7:93, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4 ISC-07/S1504 Na-type (strongly acidic cation-exchange resin of styrene-divinylbenzene copolymer with 10% crosslinkage) (Shimadzu)

Mobile phase: Gradient. A was EtOH:0.2 N pH 3.2 sodium citrate 7:93. B was 0.6 N pH 10.0 sodium citrate. C was 200 mM NaOH. A:B:C from 100:0:0 to 88:12:0 over 20 min, to 40:60:0 (step gradient), to 0:100:0 over 15 min, maintain at 0:100:0 for 5 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 5 min, re-equilibrate at initial conditions for 10 min. (Parameters are approximate.)

Column temperature: 50

Flow rate: 0.3

Detector: F ex 348 em 450 following post-column reaction. The column effluent mixed with the reagent solution pumped at 0.2 mL/min and the mixture flowed through a 200 × 0.5 stainless steel or PTFE coil at 55°. The effluent from the coil mixed with the fluorescence solution pumped at 0.2 mL/min and flowed through a 2 m × 0.5 mm stainless steel or PTFE coil at 55° to the detector. (Prepare reagent solution by adding 400 μL NaOCl solution (chlorine concentration 10%) to 1 L buffer, discard after 2 weeks. Prepare fluorescence solution by adding 15 mL EtOH containing 1.6 g o-phthalaldehyde and 2.0 g N-acetyl-L-cysteine and 4 mL 10% Brij 35 in water to 980 mL buffer, discard after 1 month. Buffer contained 384 mM sodium carbonate, 216 mM boric acid, and 108 mM potassium sulfate, pH 10.0.)

CHROMATOGRAM

Retention time: 8 (Asp), 10 (Thr), 11 (Ser), 13 (Glu), 14 (Pro), 19 (Gly), 20 (Ala), 22 (Cys), 25 (Val), 28 (Met), 30 (Ile), 31 (Leu), 33 (Tyr), 35 (Phe), 40 (His), 45 (Lys), 48 (ammonia), 54 (Arg)

Limit of quantitation: 10 pmole

KEY WORDS

derivatization; post-column reaction; soybeans

REFERENCE

Fujiwara,M.; Ishida,Y.; Nimura,N.; Toyama,A.; Kinoshita,T. Postcolumn fluorometric detection system for liquid chromatographic analysis of amino and imino acids using o-phthalaldehyde/N-acetyl-L-cysteine reagent, *Anal.Biochem.*, **1987**, *166*, 72–78.

SAMPLE

Matrix: protein

Sample preparation: Heat protein in 5.9 M HCl (twice distilled) in a sealed tube at 108° for 22 h, evaporate to dryness under reduced pressure, reconstitute with water, evaporate to dryness under reduced pressure, repeat reconstitution and evaporation step, dry over NaOH under reduced pressure overnight, reconstitute with water. Mix 100 μL of this solution (containing 10-40 μg free amino acids) with 100 μL 500 mM sodium bicarbonate solution and 100 μL freshly prepared 20 mM dansyl chloride, heat at 65° for 40 min (or let stand at room temperature for 24 h) in the dark, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS C18

Mobile phase: Gradient. A was MeCN:25 mM NaH_2PO_4 containing 25 mM acetic acid 14:86, adjusted to pH 7.00 with concentrated NaOH. B was MeCN. A:B 100:0 for 5 min, to 92:8 over 5 min, maintain at 92:8 for 15 min, to 55:45 over 30 min, to 42:58 over 5 min, re-equilibrate at initial conditions for 20 min. Every 10-15 runs wash column with 200 mM acetic acid for 40 min.

Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 8.49 (Asp), 10.13 (Glu), 16.28 (4-Hydroxyproline), 18.60 (Ser), 19.00 (His (α-derivative)), 19.70 (Arg), 20.14 (Thr), 20.59 (Gly), 22.06 (Ala), 25.18 (Pro), 31.09 (Val), 33.65 (Met), 35.49 (Ile), 35.87 (Leu), 37.85 (Phe), 38.95 (Cys (bis derivative)), 40.85 (ammonia), 44.05 (Desmosine), 44.05 (Isodesmosine), 44.46, 44.80 (5-Hydroxylysine (bis derivative)), 47.34 (Lys (bis derivative)), 49.88 (His (bis derivative)), 52.82 (Tyr (bis derivative))
Limit of quantitation: 100 pmole

KEY WORDS

derivatization

REFERENCE

Negro,A.; Garbisa,S.; Gotte,L.; Spina,M. The use of reverse-phase high-performance liquid chromatography and precolumn derivatization with dansyl chloride for quantitation of specific amino acids in collagen and elastin, *Anal.Biochem.*, **1987**, *160*, 39–46.

SAMPLE

Matrix: protein
Sample preparation: 0.1-1 μg protein + 20 μL 5.7 M HCl (twice distilled), seal under reduced pressure, heat at 110° for 18 h, dry under vacuum, add 10 μL 4 mM dabsyl chloride in acetone, heat at 70° for 10 min, dry under vacuum, reconstitute with 100-500 μL MeCN:water 50:50 over EtOH:water 70:30, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5 octadecylsilane (Nakarai Chemicals, Kyoto)
Mobile phase: Gradient. A was DMF:17 mM pH 6.5 phosphate buffer 2:98. B was MeCN:DMF 96:4. A:B from 85:15 to 55:45 over 25 min, to 30:70 over 10 min.
Flow rate: 1
Injection volume: 10
Detector: UV 420

CHROMATOGRAM

Retention time: 13.5 (Asp), 14.5 (Glu), 20 (Ser), 20.8 (Thr), 21.5 (Gly), 21.8 (Ala), 22.2 (Arg), 22.5 (Pro), 23 (Val), 24.5 (Met), 25 (Ile), 25.5 (Leu), 27 (Phe), 35 (Lys), 35.5 (His), 38 (Tyr)

KEY WORDS

derivatization

REFERENCE

Odani,S.; Kenmochi,N.; Ogata,K. A comparative study on 40S ribosomal proteins of Artemia salina and rat liver: micro analysis of amino acid composition by high-performance liquid chromatography, *J.Biochem.(Tokyo)*, **1988**, *103*, 872–877.

SAMPLE

Matrix: protein
Sample preparation: Hydrolyze 50-100 pmole protein with 200 μL 6 M HCl in a sealed argon-flushed vial at 110° for 24 h, evaporate to dryness, add 50 μL triethylamine:water 25:75, evaporate to dryness under reduced pressure, repeat this step, add 100 μL triethylamine:water 25:75, add 100 μL 1 mM Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent) in acetone, mix, shake gently in the dark at 40° for 1 h, add 20 μL 2 M HCl, dry under reduced pressure, dissolve in DMSO:water 50:50, inject an aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Nucleosil C8
Column: 250 × 4.6 5 μm Aquapore RP-300 C8
Mobile phase: Gradient. A was THF:13 mM trifluoroacetic acid 4:96. B was MeCN:THF: 13 mM trifluoroacetic acid 50:2:48. A:B from 100:0 to 65:35 over 50 min, to 60:40 (step gradient), maintain at 60:40 for 5 min, to 15:85 over 55 min.
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM

Retention time: 22.5 (cysteic acid), 35.5 (S-carboxymethylcysteine), 40 (serine), 41.5 (threonine), 43.5 (arginine), 46 (glycine), 48 (aspartic acid), 54 (glutamic acid), 54.5 (alanine), 56 (proline), 68 (histidine), 68.5 (methionine), 70 (valine), 78.5 (isoleucine), 80.5 (leucine), 82.5 (tryptophan), 84 (phenylalanine), 87 (lysine), 88.5 (cysteine), 104.5 (tyrosine)
Limit of detection: 50 pmole

KEY WORDS

derivatization; comparison with other derivatizing reagents

REFERENCE

Kochhar,S.; Christen,P. Amino acid analysis by high-performance liquid chromatography after derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, *Anal.Biochem.*, **1989**, *178*, 17–21.

SAMPLE

Matrix: protein
Sample preparation: Add 200 μL concentrated HCL containing 1% phenol to 0.1-5 μg protein, seal tube under vacuum, heat at 150° for 1 h or 108° for 24 h, evaporate to dryness under reduced pressure, add 10-20 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 20 μL reagent, let stand at room temperature for 20 min, evaporate to dryness under reduced pressure, reconstitute with PTC amino acid diluent (Waters), inject a 1-40 μL aliquot (J.Chromatogr. 1984, 336, 93). (Reagent was EtOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, store at -20°.)

HPLC VARIABLES

Column: 150 × 3.9 Nova Pak C18
Mobile phase: Gradient. A was MeCN:145 mM pH 6.2 ammonium acetate 6:94. B was MeCN:water 60:40. A:B from 100:0 to 40:60 over 15 min (Waters convex gradient 5), clean with 0:100 for 2 min.
Column temperature: 43
Flow rate: 0.7
Injection volume: 1-40
Detector: UV 254 or MS, VG Masslab Model 30-250 quadrupole, VG Masslab thermospray interface, the column effluent was combined with 200 mM ammonium acetate pumped at 0.3 mL/min and this mixture flowed into the MS, thermospray nozzle 32°, thermospray chamber 340

CHROMATOGRAM

Retention time: 1.6 (Asp), 1.9 (Glu), 4.7 (Ser), 5.0 (Gly), 6.1 (His), 8.1 (Arg), 8.7 (Thr), 9.3 (Ala), 10.1 (ammonia), 11.2 (Pro), 14.5 (Tyr), 15.4 (Val), 16.0 (Met), 16.8 (Cys), 17.3 (Ile), 17.5 (Leu), 18.6 (Phe), 20.2 (Lys)

KEY WORDS

derivatization

REFERENCE

Pramanik,B.C.; Moomaw,C.R.; Evans,C.T.; Cohen,S.A.; Slaughter,C.A. Identification of phenylthiocarbamyl amino acids for compositional analysis by thermospray liquid chromatography/mass spectrometry, *Anal.Biochem.*, **1989**, *176*, 269–277.

SAMPLE

Matrix: protein

Sample preparation: Treat 10 nmoles protein immobilized on 5 mg p-phenylenediisothiocyanate glass with 20 mg thio-acetylated glass beads (Sigma), 500 μL 10% thioacetylthioglycolic acid in MeOH, and 200 μL 20% triethylamine in MeOH with constant agitation at 45° for 30 min, wash with 2 mL 20% triethylamine in MeOH, wash with 3 mL MeOH, wash with 3 mL dichloromethane, dry under vacuum for 15 min, add 500 μL trifluoroacetic acid, let stand at room temperature for 15 min. Remove the acid, wash the solid three times with 200 μL portions of dichloromethane. Combine the acid and the washes and add 100 μL 4-nitrobenzenesulfonyl chloride (2.5 eq) in dichloromethane. Evaporate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 500 μL dichloromethane, add 50 μL 20% triethylamine in dichloromethane, concentrate immediately under reduced pressure, reconstitute, inject a 20 μL aliquot. (Synthesis of thioacetylthioglycolic acid is as follows. Reflux 8.8 g acetaldehyde, 30 mL piperidine, and 9.6 g sulfur for 1 h, cool, add water, acidify with concentrated HCl, crush the solid, wash with water, recrystallize from 200 mL EtOH to give thioacetylpiperidine (mp 55-56°). Dissolve 10 g thioacetylpiperidine in 50 mL dry benzene (Caution! Benzene is a carcinogen!), add 10.7 g bromoacetic acid, let stand at room temperature for 24 h, add 150 mL dry ether, recrystallize from EtOH/ether to give S-carboxyethylthiomethylpiperidinium bromide (mp 168-169°). Dissolve 10.1 g S-carboxyethylthiomethylpiperidinium bromide in 40 mL EtOH, cool in ice, pass hydrogen sulfide through the solution at 2-3 bubbles/s for 3-4 h (Caution! Hydrogen sulfide is highly toxic!), let stand overnight at 0°, remove EtOH by evaporation under reduced pressure, repeatedly extract the residue with ether until it is colorless. Evaporate the ether solution to dryness under reduced pressure, recrystallize from light petroleum (bp 60-100°) to obtain thioacetylthioglycolic acid (mp 80-81°) (Acta Chem. Scand. 1961, 15, 1087). 4-Acetylmorpholine can also be used as the starting reagent (Anal. Biochem. 1989, 181, 113).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm IBM C18

Mobile phase: Gradient. MeCN:MeOH:buffer from 35:0:65 to 42:0:58 over 4.9 min, to 36:9:55 over 0.1 min, to 60:15:25 over 10 min, maintain at 60:15:25 for 2 min, to 35:0:65 over 0.1 min, maintain at 35:0:65 for 3.9 min. (Prepare buffer by dissolving 10 mL acetic acid, 4 mL triethylamine, and 2 mL trifluoroacetic acid, pH 3.1.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 248

CHROMATOGRAM

Retention time: 3.2 (Asn), 3.7 (Gln), 4.6 (Asp), 5.0 (Arg), 5.9 (Glu), 8.3 (Gly), 9.3 (Ala), 10.0 (Lys), 12.6 (Met), 13.4 (Val), 13.9 (Trp), 14.7 (Phe), 15.0 (Leu, Ile), 17.0 (Tyr)

KEY WORDS

derivatization

REFERENCE

Stolowitz,M.L.; Paape,B.A.; Dixit,V.M. Thioacetylation method of protein sequencing: derivatization of 2-methyl-5(4*H*)-thiazolones for high-performance liquid chromatographic detection, *Anal.Biochem.*, **1989**, *181*, 113–119.

SAMPLE

Matrix: protein

Sample preparation: Hydrolyze protein with 80 μL 3% phenol in 6 M HCl in an evacuated tube at 166° for 25 min, evaporate to dryness under reduced pressure at 50°, reconstitute with 10 μL 200 mM pH 9.0 carbonate buffer, add 20 μL 4 mM dabsyl chloride in MeCN, heat at 70° with occasional shaking for 10 min, dilute to 200 μL with MeCN:water 66:34, centrifuge, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Hypersil ODS
Mobile phase: Gradient. A was acetone:10 mM pH 6.5 sodium phosphate buffer 7.5:92.5. B was acetone:10 mM pH 6.5 sodium phosphate buffer 50:50. A:B from 100:0 to 0:100 over 40 min. (Place a 50 × 4.6 column of 30 μm Wakogel LC ODS-30K before the injector.)
Column temperature: 60
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM

Retention time: 11 (aspartic acid), 12 (glutamic acid), 18.5 (serine), 19.3 (threonine), 19.7 (glycine), 20.5 (alanine), 21.5 (proline), 23 (valine), 24 (arginine), 25 (methionine), 26 (isoleucine), 26.5 (leucine), 27 (tryptophan), 27.5 (phenylalanine), 30 (cysteine), 38.5 (lysine), 39.5 (histidine), 43 (tyrosine)

KEY WORDS

derivatization

REFERENCE

Muramoto,K.; Kamiya,H. Recovery of tryptophan in peptides and proteins by high-temperature and short-term acid hydrolysis in the presence of phenol, *Anal.Biochem.*, **1990**, *189*, 223–230.

SAMPLE

Matrix: protein
Sample preparation: Dissolve 1 mg protein in 2 mL 6 M HCl, heat in a sealed tube at 90° for 12 h, evaporate to dryness under reduced pressure, reconstitute with 2 mL water. Mix 25 μL hydrolyzate, 25 μL pH 9.1 saturated sodium borate, 25 μL 20 mM cetyltrimethylammonium bromide, 12.5 μL MeCN, and 10 mg polymeric reagent, heat at 70° for 10 min, elute with 1 mL MeCN:water 70:30, inject a 20 μL aliquot of the eluate. (Prepare polymeric reagent as follows. Soxhlet extract styrene-divinylbenzene copolymer (12% cross-linked, 60 Å templated, 10-20 μm, Supelco) with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

HPLC VARIABLES
Column: 250 × 4.6 YMC AP-303 300 Å ODS (YMC)
Mobile phase: Gradient. A was 0.05% trifluoroacetic acid in water. B was 0.05% trifluoroacetic acid in MeCN. A:B from 70:30 to 30:70 over 16 min, maintain at 30:70 for 6 min, return to initial conditions over 30 s.
Flow rate: 1.5
Injection volume: 20
Detector: F ex 254 em 305-395

CHROMATOGRAM
Retention time: 5.8 (His), 6.3 (Asn, Gln), 7.2 (Ser), 7.4 (Glu), 7.8 (Thr), 9.1 (Ala), 10.6 (Pro), 11 (Met), 12.2 (Tyr), 12.6 (Ile), 12.8 (Leu), 13 (Phe), 14.6 (Lys)

KEY WORDS
derivatization

REFERENCE
Zhou,F.-X.; Krull,I.S.; Feibush,B. Solid-phase derivatization of amino acids and peptides in high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *648*, 357–365.

SAMPLE
Matrix: protein
Sample preparation: Heat 50 μg protein with 200 μL 6 M HCl and 1 crystal of phenol at 153° for 1 h, evaporate to dryness under reduced pressure, reconstitute with water. Mix 25 μL hydrolyzate, 25 μL saturated sodium borate, 25 μL 20 mM cetyltrimethylammonium bromide, 12.5 μL MeCN, and 15 mg polymeric 6-aminoquinoline reagent, heat at 70° for 10 min, elute with 1 mL MeCN:water 70:30, inject a 20 μL aliquot of the eluate. (Prepare polymeric 6-aminoquinoline reagent as follows. React 10 g 4-chloro-3-nitrobenzyl alcohol, 10 g dried 16-20 μm styrene-divinylbenzene copolymer (12% cross-linked, 102 Å templated, Supelco), and 10 g anhydrous aluminum chloride in 50 mL nitrobenzene at 65-70° for 3 days, cool to room temperature, filter, wash with three 50 mL portions of 1 M HCl in dioxane (Caution! Dioxane is a carcinogen!), wash with three 50 mL portions of DMF, wash with three 50 mL portions of MeOH, wash with three 50 mL portions of dichloromethane, dry under reduced pressure at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:2-ethoxyethanol 40:60 for 20 h, cool to room temperature, filter, wash thoroughly with water, suspend the polymer in 100 mL concentrated aqueous HCl: dioxane 50:50, reflux for 20 h, filter, wash with five 100 mL portions of water, wash with three 100 mL portions of MeOH, wash with three 50 mL portions of ether, dry under reduced pressure at 80° to obtain polymeric benzotriazole reagent (Eur. J. Biochem. 1975, 59, 55). Heat 1.6 g quinoline-6-carboxylic acid (ICN Biomedicals, Costa Mesa CA) with 2.7 mL thionyl chloride in 55 mL dry benzene at 65-75° for 1 h (Caution! Benzene is a carcinogen!), remove the benzene by evaporation under reduced pressure, add 21 mL acetic acid, stir strongly for several min, slowly add 600 mg sodium azide at room temperature, stir for 1 h, add 53 mL triethylamine, add 100 mL water, stir for 30 min, filter, wash ten times with 25 mL portions of water, dry under vacuum overnight to obtain quinoline-6-acyl azide. Heat 3.3 g polymeric benzotriazole reagent and 2 g quinoline-6-acyl azide in 150 mL toluene (dried over calcium hydride) at 60-65° for 2 h, wash with 600 mL warm (40°) dichloromethane, wash with 50 mL MeCN, dry under vacuum overnight to obtain polymeric 6-aminoquinoline reagent.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 140 mM sodium acetate containing 17 mM triethylamine, adjusted to pH 5.05 with phosphoric acid. B was MeCN:water 60:40. A:B from 100:0 to 50:50 over 30 min, to 0:100 (step gradient), maintain at 0:100 for 5 min, return to initial conditions, re-equilibrate at initial conditions for 10 min.
Flow rate: 1.5
Injection volume: 20

Detector: F ex 254 em 395

CHROMATOGRAM
Retention time: 15-30 (depending on structure, peaks not identified)
Limit of detection: 1 ppm

KEY WORDS
derivatization

REFERENCE
Yu,J.H.; Li,G.D.; Krull,I.S.; Cohen,S. Polymeric 6-aminoquinoline, an activated carbamate reagent for derivatization of amines and amino acids by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 249–260.

SAMPLE
Matrix: protein
Sample preparation: Heat 300 mg protein and 1.5 mL 6 M HCl at 110° in a sealed tube for 24 h, evaporate to dryness. Dissolve an amount of protein hydrolysate containing 4 mmole amino acids in 2 mL 2 M NaOH, stir at 0°, add 1.36 g benzyl chloroformate and 250 μL 4 M NaOH simultaneously over 40 min so as to maintain the pH between 10 and 12, wash the reaction mixture with 250 μL pentane. Cool the aqueous layer and acidify to Congo red with 4 M HCl, extract with three 200 μL portions of ethyl acetate. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, reconstitute with MeOH, inject a 10 μL MeOH.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Econosil C18
Mobile phase: Gradient. A was 0.5% trifluoroacetic acid in water. B was 0.5% trifluoroacetic acid in MeCN. A:B from 100:0 to 40:60 over 1 h, to 0:100 over 5 min, maintain at 0:100 for 5 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 17 (Asn), 18 (Gln), 18.5 (His), 19 (Ser), 20 (Thr), 22 (Asp), 23 (Glu), 24 (Gly), 28 (Ala), 33 (Pro), 34 (Tyr), 39 (Val), 42 (Met), 45.5 (Leu), 45.5 (Ile), 45.5 (Trp), 46 (Phe), 49.5 (Lys), 56 (Cys), 58 (Arg)

KEY WORDS
derivatization

REFERENCE
Egorova,T.A.; Eremin,S.V.; Mitsner,B.I.; Zvonkova,E.N.; Shvets,V.I. Isolation of individual amino acids from various microbiological sources using reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 53–62.

SAMPLE
Matrix: protein
Sample preparation: Heat 2 mg protein and 1 mL 6 M HCl in a sealed tube at 110° for 20 h, reconstitute with 5 mL buffer. Remove a 400 μL aliquot and add it to 600 μL buffer and 400 μL 2 mM 9-isothiocyanatoacridine in MeCN, heat at 60° with occasional shaking for 1.5 h, cool, inject a 20 μL aliquot. (Prepare buffer by mixing 500 mL 100 mM boric acid containing 100 mM KCl with 408 mL 100 mM NaOH, make up to 1 L with water, pH 9.8. Prepare 9-isothiocyanatoacridine as follows. Reflux 15 mmoles 9-chloroacridine (Eastman) and 16 mmoles silver thiocyanate (Aldrich) in 150 mL anhydrous toluene, recrystallize 9-isothiocyanatoacridine from anhydrous acetone, mp 131-2° (Chem.Abs. 1970, 72, 21584v).)

HPLC VARIABLES
Column: 150 × 3.3 5 μm Separon C-18 SGX (Tessek, Prague)
Mobile phase: MeCN:23 mM pH 6 ammonium formate 20:80
Flow rate: 0.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.62 (Glu), 4.85 (Asp), 5.88 (Ala), 6.44 (Asn), 6.83 (Gly), 7.10 (Ser), 7.90 (Thr), 8.37 (His), 10.73 (Gln), 11.63 (Val), 12.27 (Tyr), 13.37 (Pro, Lys), 15.20 (Arg), 16.97 (Met), 26.07 (Ile), 26.60 (Leu), 36.93 (Phe), 38.97 (Trp)
Limit of detection: 100-400 pmole

KEY WORDS
derivatization

REFERENCE
Oravec,P.; Podhradsky,D. High-performance liquid chromatography of amino acids after derivatization with 9-isothiocyanatoacridine, *J.Biochem.Biophys.Methods*, **1995**, *30*, 145–152.

SAMPLE
Matrix: protein
Sample preparation: For N-terminal amino acid. Add 200 pmole protein and 200 pmole nor-leucine to a tube, dry under reduced pressure, dissolve in 40 μL pH 9.5 lithium carbonate buffer, add 20 μL 1.5 mg/mL dansyl chloride in MeCN, shake for 2 min, heat at 37° for 40 min, add 2 μL 8.9 M ethylamine hydrochloride, heat at 37° for 10 min, evaporate to dryness under reduced pressure at room temperature, reconstitute with 100 μL constant boiling HCl, heat at 110° for 18 h, evaporate to dryness over solid NaOH, reconstitute with 100 μL initial mobile phase, inject an aliquot. For amino acid mixtures. Add 20 nmole amino acid mixture to a tube, dry under reduced pressure, dissolve in 40 μL pH 9.5 lithium carbonate buffer, add 20 μL 1.5 mg/mL dansyl chloride in MeCN, shake for 2 min, heat at 37° for 40 min, add 2 μL 8.9 M ethylamine hydrochloride, heat at 37° for 10 min, evaporate to dryness under reduced pressure at room temperature, reconstitute with 100 μL initial mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 50 mm long 37-50 μm C18/Corasil
Column: 150 × 3.9 4 μm NovaPak C18
Mobile phase: Gradient. A was MeOH:THF:100 mM pH 7.4 sodium phosphate buffer 5:6.5:88.5. B was MeOH:water 70:30. A:B from 100:0 to 0:100 over 30 min (Waters curve no.6), return to initial conditions over 10 min, re-equilibrate at initial conditions for 10 min. (At the end of each day wash column with 35 mL water and 20 mL MeOH:water 70:30.)
Column temperature: 25
Flow rate: 1
Detector: F ex 338 (bandpass filter) ex 455 (long-pass filter)

CHROMATOGRAM
Retention time: 4.5 (Asp), 5.5 (Glu), 12 (Ser), 12.5 (Arg), 13 (Thr), 14 (Gly), 14.7 (Ala), 15 (Pro), 18.5 (Val), 19 (ammonia), 20 (Met), 21.5 (Ile), 22 (Leu), 23.5 (Phe), 25 (ethylamine), 26 (cystine, bis-derivative), 29 (Lys, bis-derivative), 30.5 (His, bis-derivative), 31 (Tyr, bis-derivative)
Internal standard: norleucine (23)
Limit of detection: 2 pmole

KEY WORDS
derivatization

REFERENCE

Martins,A.R.; Padovan,A.P. A practical approach to improve the resolution of dansyl-amino acids by high-performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 467–476.

SAMPLE

Matrix: protein

Sample preparation: Hydrolyze protein with 1 mL 6 M HCl for each 1 mg protein, heat under nitrogen at 110° for 24 h, filter (paper), make up the filtrate to 250 mL, evaporate to dryness under reduced pressure at 40°, reconstitute the residue with water so as to give a protein concentration of 150-250 μg/mL. 1 mL Hydrolysate + 2 mL 40 mM pH 9.5 lithium carbonate + 1 mL 4 mg/mL dansyl chloride in MeCN, mix, heat at 60° for 30 min, add 50 μL 2% methylamine in water, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm Spherisorb ODS-2
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 39:61
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.2 (lysine, bis derivative), 4.7 (histidine, bis derivative), 6.7 (ammonia), 11.7 (tyrosine), 15 (methylamine)

KEY WORDS

casein; lysozyme; lentils; enteral solution; derivatization

REFERENCE

Sanz,M.A.; Castillo,G.; Hernández,A. Isocratic high-performance liquid chromatographic method for quantitative determination of lysine, histidine and tyrosine in foods, *J.Chromatogr.A*, **1996**, *719*, 195–201.

SAMPLE

Matrix: protein hydrolysate

Sample preparation: 2-200 μg Protein hydrolysate + 1 mL 1 M pH 9.0 sodium borate buffer containing 0.02% sodium azide + 0.8 μL diethyl ethoxymethylenemalonate (Fluka), heat at 50° with vigorous shaking for 50 min, cool to room temperature, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. MeCN:buffer from 9:91 to 14:86 over 3 min, maintain at 14:86 for 10 min, to 31:69 over 17 min, maintain at 31:69 for 5 min. (Buffer was 25 mM pH 6.0 sodium acetate containing 0.02% sodium azide. Caution! Sodium azide is highly toxic! Do not discharge solutions containing sodium azide to the plumbing system!)

Column temperature: 18
Flow rate: 0.9
Injection volume: 15
Detector: UV 280

CHROMATOGRAM

Retention time: 3.10 (aspartic acid), 3.31 (glutamic acid), 7.48 (serine), 9.35 (histidine), 10.25 (glycine), 11.02 (threonine), 12.66 (arginine), 13.45 (alanine), 15.25 (proline), 20.05 (tyrosine), 24.21 (ammonia), 25.84 (valine), 27.22 (methionine), 28.52 (cystine), 30.19 (isoleucine), 31.02 (leucine), 31.95 (phenylalanine), 34.73 (lysine)

Internal standard: α-aminobutyric acid (18.15)

Limit of detection: 3 pmole

KEY WORDS
derivatization

REFERENCE
Alaiz,M.; Navarro,J.L.; Girón,J.; Vioque,E. Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate, *J.Chromatogr.*, **1992**, *591*, 181–186.

SAMPLE
Matrix: protein hydrolysate, exopeptidase digest
Sample preparation: 5-10 μL Protein hydrolysate or exopeptidase digest + 40 μL MeOH:triethylamine 8:1 + 5 μL phenylisothiocyanate, mix, let stand at room temperature for 20 min, wash twice with 15 μL portions of heptane, dilute the aqueous phase with 40 μL 50 mM pH 5.4 sodium acetate buffer:acetic acid 100:3, let stand for 30 s, add 40 μL 50 mM pH 5.4 sodium acetate buffer:acetic acid 100:3, let stand for 30 s, inject a 90 μL aliquot.

HPLC VARIABLES
Mobile phase: Gradient. A was 50 mM pH 5.4 sodium acetate. B was MeCN:water 70:30. A:B from 93:7 to 70:30 over 10 min, to 42:58 over 10 min.
Column temperature: 37
Flow rate: 0.3
Injection volume: 90
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5 (Asp), 6 (Glu), 6.7 (Ser), 7.2 (Gly), 7.5 (His), 8 (Arg), 8.5 (Thr), 9 (Ala), 9.3 (Pro), 11.6 (Tyr), 12.6 (Val), 13 (Met), 13.7 (Cystine), 14.8 (Ile), 15 (Leu), 15.4 (Norleucine), 15.8 (Phe), 17 (Lys)
Limit of quantitation: 50 pmole

KEY WORDS
derivatization

REFERENCE
Thoma,R.S.; Crimmins,D.L. Automated phenylthiocarbamyl amino acid analysis of carboxypeptidase/aminopeptidase digests and acid hydrolysates, *J.Chromatogr.*, **1991**, *537*, 153–165.

SAMPLE
Matrix: shrimp
Sample preparation: Blend (Waring) 1 g shrimp and 2 mL trichloroacetic acid, centrifuge, mix an aliquot of the supernatant with equal volumes of 400 mM borate buffer and 2 mg/mL 4-chloro-7-nitrobenzofurazan in MeOH, heat at 60° for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Lichrosorb RP-C18
Mobile phase: Gradient. A was THF:100 mM sodium acetate buffer 1:99, pH 6.2. B was MeOH. A:B from 80:20 to 70:30 over 5 min, maintain at 70:30 for 3 min, to 50:50 over 1.5 min, maintain at 50:50 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.2
Injection volume: 10
Detector: F ex 220 em 370 (filter)

CHROMATOGRAM
Retention time: 4.5 (hydroxyproline), 8.9 (proline)

KEY WORDS
derivatization

REFERENCE
Vázquez-Ortiz,F.A.; Caire,G.; Higuera-Ciapara,I.; Hernández,G. High performance liquid chromatographic determination of free amino acids in shrimp, *J.Liq.Chromatogr.*, **1995**, *18*, 2059–2068.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 6μm Zorbax TMS
Mobile phase: 1.8 mM pH 4.1 copper sulphate (Prepare mobile phase by dissolving 450 mg copper sulfate pentahydrate in 1 L water and adjusting the pH to 4.1 with 50 mM sulfuric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 3.517 (glutamic acid), 4.142 (threonine), 5.007 (ornithine), 5.812 (histidine), 6.887 (arginine), 10.425 (phenylalanine)

REFERENCE
Khedr,A. High-performance liquid chromatography of α-amino acids and aztreonam on reversed phase columns with aqueous Cu2+ as eluent, *Biomed.Chromatogr.*, **1996**, *10*, 167–171.

SAMPLE
Matrix: solutions
Sample preparation: 20-100 μL Amino acid solution in water + 20-100 μL pH 9.0 Titrisol buffer (Merck) + 20-100 μL 2.7 mg/mL dansyl chloride in water-free acetone, let stand in the dark at room temperature for 1 h, evaporate to dryness under reduced pressure, reconstitute with 100 μL acetone:1 M HCl 95:5, inject a 0.1-1 μL aliquot.

HPLC VARIABLES
Column: 500 × 3 5 μm LiChrosorb SI 60
Mobile phase: Gradient. A was benzene:pyridine:acetic acid 100:10:1. B was pyridine:acetic acid 90:9. A:B from 100:0 to 0:100 over 25 min in a complex fashion. The gradient was obtained by adding 15 mL B over 15 min to a reservoir containing 50 mL A, then adding 15 mL B over 5 min. While these additions were happening the mobile phase was pumped out of the reservoir at 1 mL/min. Re-equilibrate at initial conditions for 5 min before the next injection. (Caution! Benzene is a carcinogen!)
Column temperature: 65
Flow rate: 1
Injection volume: 0.1-1
Detector: F ex 340 em 510

CHROMATOGRAM
Retention time: 4 (isoleucine), 5 (leucine), 6 (valine), 7.5 (proline), 10 (phenylalanine), 12 (methionine), 13 (alanine), 14 (lysine, didansyl), 15 (tyrosine), 17 (glycine), 18 (tryptophan), 19 (glutamic acid), 20 (threonine), 22 (serine), 23 (aspartic acid), 2.5 (cystine, mobile phase benzene:pyridine:acetic acid:MeOH 100:50:0.5:50), 3 (histidine, mobile phase benzene:pyridine:acetic acid:MeOH 100:50:0.5:50), 5 (arginine, mobile phase benzene:pyridine:acetic acid:MeOH 100:50:0.5:50)

KEY WORDS
derivatization; normal phase

REFERENCE

Bayer,E.; Grom,E.; Kaltenegger,B.; Uhmann,R. Separation of amino acids by high performance liquid chromatography, *Anal.Chem.*, **1976**, *48*, 1106–1109.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 50 μL aliquot of a 1 mg/mL amino acid solution in MeCN: triethylamine 99.8:0.2 with 50 μL 0.5% 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in MeCN, let stand at room temperature for 1 h, inject a 5 μL aliquot. (2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate may be purchased from Aldrich, synthesis details are also given in this paper.)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-18

Mobile phase: MeOH:water 50:50 (A) or 60:40 (B)

Flow rate: 0.4

Injection volume: 5

Detector: UV 250

CHROMATOGRAM

Retention time: 10 (D-Ser (A)), 11 (L-Ser (A)), 14 (L-Tyr (B)), 15 (L-Ala (A)), 16 (D-Ala (A), D-Tyr (B)), 19 (L-Val (B)), 21 (D-Val (B)), 22 (L-phenylglycine (B)), 23 (D-phenylglycine (B)), 25 (L-Asp (A)), 28 (L-Leu (B)), 28.5 (D-Asp (A), L-Glu (A)), 30 (D-Glu (A)), 32 (D-Leu (B), L-Phe (B)), 38 (D-Phe (B))

Limit of detection: 5 ng

KEY WORDS

derivatization; chiral

REFERENCE

Nimura,N.; Ogura,H.; Kinoshita,T. Reversed-phase liquid chromatographic resolution of amino acid enantiomers by derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, *J.Chromatogr.*, **1980**, *202*, 375–379.

SAMPLE

Matrix: solutions

Sample preparation: Rapidly mix 2 mL 0.001-1 mM amino acid solution in buffer with 1 mL 1.5 mg/mL dansyl chloride in MeCN, shake gently for 2 min, let stand at room temperature for 35 min, add 100 μL 2% ethylamine hydrochloride, inject a 25 μL aliquot. (Buffer was 40 mM lithium carbonate adjusted to pH 9.5 with HCl. Distil MeCN from dansyl chloride to remove trace impurities. Protect reaction from light.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil C8

Mobile phase: MeOH:buffer 42:58 (Buffer was 0.6% glacial acetic acid containing 0.008% triethylamine.)

Flow rate: 2

Injection volume: 25

Detector: F ex 250 em 470

CHROMATOGRAM

Retention time: 2.5 (Asn), 3.5 (ammonia), 4 (Asp), 5.5 (Thr), 7 (Ala), 17 (Met)

Limit of detection: 1 pmole

KEY WORDS

derivatization

REFERENCE

Tapuhi,Y.; Schmidt,D.E.; Lindner,W.; Karger,B.L. Dansylation of amino acids for high-performance liquid chromatography analysis, *Anal.Biochem.*, **1981**, *115*, 123–129.

SAMPLE

Matrix: solutions

Sample preparation: 1 mL Solution + 3 mL 200 mM pH 9.0 borate buffer + 1 mL 30 mM 2,4,6-trinitrobenzenesulfonic acid in water, let stand at room temperature for 1 h, dilute, inject an aliquot. Alternatively, acidify reaction mixture with 1 drop 6 M HCl, add 2.5 mL hexane:ethyl acetate 20:80, vortex for 15 s. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL 100 mM perchloric acid, dilute 1:200, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Biophase 6032 octyl (Bioanalytical Systems)

Mobile phase: n-Propanol:100 mM pH 5.0 sodium acetate 12.5:87.5

Flow rate: 1

Detector: E, Bioanalytical Systems LC-154, TL-5A glassy carbon electrode -0.85 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.5 (glutamic acid), 16 (serine), 17 (threonine), 18 (glycine), 19.5 (alanine)

KEY WORDS

derivatization

REFERENCE

Jacobs,W.A.; Kissinger,P.T. Nitroaromatic reagents for determination of amines and amino acids by liquid chromatography/electrochemistry, *J.Liq.Chromatogr.*, **1982**, *5*, 881–895.

SAMPLE

Matrix: solutions

Sample preparation: Mix 400 μL aqueous sample solution with 100 μL buffer and 500 μL 15 mM fluorenylmethyl chloroformate in acetone, let stand for 40 s, wash three times with 2 mL portions of pentane, inject an aliquot of the aqueous layer. (Prepare buffer by adjusting the pH of 1 M boric acid to 6.2 with NaOH.)

HPLC VARIABLES

Column: 125 × 4.6 3 μm ODS Hypersil

Mobile phase: Gradient. MeCN:MeOH:buffer 10:40:50 for 3 min, to 50:0:50 over 9 min.

Flow rate: 1.3 for 12 min, to 2 over 0.5 min

Injection volume: 10

Detector: F ex 250 (filter) em 320 (filter)

CHROMATOGRAM

Retention time: 4.5 (Asn), 4.8 (Gln), 5 (Asp), 5.5 (Ser), 6 (Glu), 7.5 (Gly), 8 (Thr), 8.7 (Arg), 10 (Ala), 10.5 (Tyr), 12.5 (Pro), 13 (Met), 14 (Val), 14.5 (Phe), 14.8 (Ile), 15 (Leu), 17.5 (His), 18 (Orn), 19.5 (Lys)

Limit of quantitation: 100 nM

KEY WORDS

derivatization

REFERENCE

Einarsson,S.; Josefsson,B.; Lagerkvist,S. Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *282*, 609–618.

SAMPLE

Matrix: solutions

Sample preparation: Mix amino acid solution with 2 mL freshly-prepared buffer, add 5 mL 10 ppm p-N,N-dimethylaminophenylisothiocyanate in acetone, purge tube with nitrogen, heat at 50° for 20 min, add 5 mL acid solution, purge with nitrogen, heat at 50° for 20 min, let stand for 20 min, evaporate to dryness under reduced pressure, wash the residue several times with isooctane, reconstitute with 100 mM pH 2 phosphate buffer, inject a 10 μL aliquot. (Prepare the buffer by mixing 50 mL water, 50 mL acetone, 5 mL 200 mM acetic acid, 1 mL triethylamine, and enough pyridine to adjust the pH to 9.5. Prepare the acid solution by mixing equal volumes of water and glacial acetic acid saturated with HCl. Synthesis of p-N,N-dimethylaminophenylisothiocyanate is as follows. Add 150 mL water containing 33 g sodium carbonate and 24 g thiophosgene to 60 mmoles N,N-dimethyl-1,4-phenylenediamine (p-N,N-dimethylaminoaniline) in 100 mL EtOH:water 50:50 with vigorous stirring, stir at room temperature for 2 h, extract with dichloromethane. Dry the organic layer and evaporate it to dryness under reduced pressure to obtain p-N,N-dimethylaminophenylisothiocyanate as an oil.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm RP-8 (Hewlett-Packard)

Mobile phase: MeCN:18.13 g/L KH_2PO_4 25:75, adjusted to pH 2 with sulfuric acid or KOH

Flow rate: 2.5

Injection volume: 10

Detector: E, Bioanalytical Systems LC-4, TL-5 glassy carbon flow-cell 0.85 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4 (Cys), 5 (Asp), 6 (CysCys), 7 (Asn), 9.5 (Ser), 10 (Glu), 10.5 (Gln), 15 (Thr), 18 (Gly), 19 (His), 20 (Ala), 22 (Arg), 25 (Met), 29 (Pro), 34 (Val), 42 (Trp), 49 (Phe), 53 (Tyr), 61 (Lys), 70 (Ile), 74 (Leu)

Limit of detection: 0.2-0.6 ng

KEY WORDS

derivatization

REFERENCE

Mahachi,T.J.; Carlson,R.M.; Poe,D.P. p-N,N-Dimethylaminophenylisothiocyanate as an electrochemical label for high-performance liquid chromatographic determination of amino acids, *J.Chromatogr.*, **1984**, *298*, 279–288.

SAMPLE

Matrix: solutions

Sample preparation: Add 50 μL of a 50 mM solution in water to 100 μL 1% Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's Reagent) in acetone, mix, add 20 μL 1 M sodium bicarbonate, mix at 30-40° for 1 h, cool to room temperature, add 10 μL 2 M HCl, mix, dry in a vacuum desiccator over NaOH, reconstitute with 500 μL DMSO, inject a 5-10 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 C18 (Waters)

Mobile phase: Gradient. MeCN:50 mM pH 3.0 triethylammonium phosphate from 10:90 to 50:50 over 1 h.

Flow rate: 2

Injection volume: 5-10

Detector: UV 340

CHROMATOGRAM

Retention time: 17.68 (L-Asp), 19.40 (L-Glu), 20.28 (D-Asp), 21.40 (L-Ala), 22.71 (D-Glu), 26.72 (D-Ala), 28.21 (L-Met), 34.66 (D-Met), 35.82 (L-Phe), 41.22 (D-Phe)

KEY WORDS
derivatization; chiral

REFERENCE
Marfey,P. Determination of D-amino acids. II. Use of a bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene, *Carlsberg Res.Commun.*, **1984**, *49*, 591–596.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate a 500 μL aliquot of an aqueous solution containing 25 μmoles amino acids to dryness under a stream of air at 37°, reconstitute with 250 μL 1 M sodium bicarbonate solution, add 500 μL 1% 2,4-dinitrofluorobenzene in acetone, mix, heat at 50° for 1 h, cool to room temperature, add 200 μL 2 M HCl, dry in a vacuum desiccator over NaOH, reconstitute in 1 mL MeOH, pass dry HCl gas through the solution for 5 min, cap the vial, let stand for 3 h, evaporate to dryness, dry in a vacuum desiccator over NaOH, reconstitute in 400 μL DMSO. Remove a 50 μL aliquot and add it to 200 μL 50 mM pH 7.5 HEPES buffer, add 50 μL 10.6 mg/mL carboxypeptidase-Y (Carlsberg Biotechnology) in water, mix, let stand at room temperature for 3 h, add 300 μL DMSO, inject a 25 μL aliquot. (All amino acids are converted to their dinitrophenyl methyl ester derivatives. The enzyme hydrolyses only L-amino acid methyl esters (at the α-position) and so D-amino acids (methyl ester at the α-position) can be distinguished from L-amino acids (carboxylic acid at the α-position). Methyl esters of carboxylic acid groups other than α are not affected. No racemization occurs during derivatization and so the procedure can be used to assess racemization reactions.)

HPLC VARIABLES
Column: 100 × 8 radial compression C18 (Waters)
Mobile phase: Gradient. MeCN:50 mM pH 3.0 triethylammonium phosphate buffer from 25:75 to 50:50 over 40 min.
Flow rate: 2
Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 8.10 (β-methoxy-L-Asp), 8.76 (L-Ala), 9.93 (gamma-methoxy-L-Glu), 15.93 (L-Met), 20.91 (dimethoxy-D-Asp), 22.75 (methoxy-D-Ala), 23.78 (L-Phe), 24.68 (dimethoxy-D-Glu), 32.58 (methoxy-D-Met), 39.59 (methoxy-D-Phe)

KEY WORDS
derivatization; chiral

REFERENCE
Marfey,P.; Ottesen,M. Determination of D-amino acids. I. Hydrolysis of DNP-L-amino acid methyl esters with carboxypeptidase-Y, *Carlsberg Res.Commun.*, **1984**, *49*, 585–590.

SAMPLE
Matrix: solutions
Sample preparation: 20 μL Amino acid solution in 100 mM HCl + 30 μL 300 mM pH 9.5 borate buffer + 50 μL 5 mM reagent in MeCN, let stand for 5 min, inject a 5 μL aliquot. (Prepare reagent as follows. Add 40 mmoles p-bromoaniline in 40 mL MeCN dropwise with stirring to 50 mmoles N,N'-disuccinimidyl carbonate in 60 mL MeCN over 3-4 h, stir for 1 h, remove the MeCN by evaporation. Dissolve the residue in 100 mL ethyl acetate, wash with 1 M HCl, wash with 4% sodium bicarbonate, wash with water, dry over anhydrous sodium carbonate, evaporate to obtain succinimido p-bromophenylcarbamate, recrystallize form benzene/acetone (Caution! Benzene is a carcinogen!)

HPLC VARIABLES
Column: 150 × 4.6 Develosil ODS-5 (Nomura Chemical)

Mobile phase: MeOH:0.1% phosphoric acid 45:55
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 7 (His), 8 (Arg), 16 (Ser), 21.5 (Pro), 26 (Thr), 28 (Ala)
Limit of detection: 0.15-0.3 ng

OTHER SUBSTANCES
Also analyzed: tert-butylamine, diethylamine, diisobutylamine, diisopropylamine, isobutylamine (mobile phase MeCN:water 55:45)

KEY WORDS
derivatization

REFERENCE
Nimura,N.; Iwaki,K.; Kinoshita,T.; Takeda,K.; Ogura,H. Activated carbamate reagent as derivatizing agent for amino compounds in high-performance liquid chromatography, *Anal.Chem.*, **1986**, *58*, 2372–2375.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL 5 mM amino acid solution with 50 μL 400 mM pH 9.0 sodium borate buffer, add 100 μL 16 mg/mL (+)-naphthylethyl isocyanate in dry acetone while vortexing, vortex for 30 s, let stand at room temperature for 5 min, centrifuge at 4000 g for 2 min. Remove a 150 μL aliquot of the supernatant and wash it three times with 1 mL portions of wash solution, inject an aliquot. (Prepare wash solution by mixing equal volumes of cyclohexane saturated with 400 mM pH 9.0 borate buffer and ether. Wash ether with ferrous sulfate to remove peroxides if methionine is present.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm octadecyl Si100 Polyol (Serva)
Mobile phase: MeCN:50 mM pH 6.2 ammonium acetate 15:85 (A) or 20:80 (B) or 25:75 (C) or 27.5:72.5 (D) or 35:65 (E) or 40:60 (F) or MeCN:100 mM acetic acid 20:80 (G)
Flow rate: 1
Detector: F ex 214 em 320 (cut-off filter)

CHROMATOGRAM
Retention time: 22.6 (D-Asp (G)), 24.4 (L-Asp (G)), 26.6 (D-Glu (G)), 28.2 (L-Glu (G)), 20.8 (D-Asn (A)), 23.2 (L-Asn (A)), 19.3 (D-Ser (A)), 20.8 (L-Ser (A)), 27.6 (D-His (A)), 31.5 (L-His (A)), 9.9 (D-Thr (B)), 11.9 (L-Thr (B)), 11.0 (D-Pro (B)), 12.2 (L-Pro (B)), 11.7 (D-Ala (B)), 13.2 (L-Ala (B)), 6.9 (D-Arg (C)), 7.7 (L-Arg (C)), 10.4 (D-Val (C)), 13.2 (L-Val (C)), 5.8 (D-Met (D)), 7.4 (L-Met (D)), 9.7 (D-Ile (D)), 12.1 (L-Ile (D)), 11.0 (D-Leu (D)), 13.5 (L-Leu (D)), 16.0 (D-Trp (D) (UV 215)), 18.3 (L-Trp (D) (UV 215)), 17.3 (D-Phe (D)), 20.6 (L-Phe (D)), 15.5 (D-Lys (E)), 17.8 (L-Lys (E)), 17.3 (D-Tyr (F)), 19.0 (L-Tyr (F))
Limit of detection: <1 pmole

KEY WORDS
derivatization; chiral

REFERENCE
Dunlop,D.S.; Neidle,A. The separation of D/L amino acid pairs by high-performance liquid chromatography after precolumn derivatization with optically active naphthylethyl isocyanate, *Anal.Biochem.*, **1987**, *165*, 38–44.

SAMPLE
Matrix: solutions

Sample preparation: Mix a 20 μL aliquot of a solution in 100 mM HCl with 20 μL 500 mM pH 9.5 borate buffer and 20 μL 1.5 mg/mL reagent, mix for 1.5 min, let stand for 3 min, inject a 6 μL aliquot. (Prepare the reagent, succinimido naphthylcarbamate, as follows. Add 40 mL 1 M 1-naphthylamine in MeCN dropwise to 60 mL MeCN containing 50 mmoles N,N'-disuccinimidyl carbonate with stirring at room temperature over 3-4 h, after addition is complete stir for 1 h at room temperature, evaporate to dryness, dissolve the residue in 100 mL ethyl acetate. Wash the solution with 1 M HCl, wash with 4% sodium bicarbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness, recrystallize from benzene/acetone to obtain succinimido naphthylcarbamate (Caution! Benzene is a carcinogen!) (Anal.Chem. 1986, 58, 2372).)

HPLC VARIABLES

Column: 10 × 6 Develosil ODS-5
Mobile phase: Gradient. MeCN:100 mM pH 6.30 sodium acetate from 14:86 to 16:84 over 5 min, to 25:75 over 10 min, to 39:61 over 6 min, to 70:30 (step gradient), maintain at 70:30 for 6 min, re-equilibrate at initial conditions for 13 min.
Column temperature: 30
Flow rate: 1.2
Injection volume: 6
Detector: F ex 290 em 370

CHROMATOGRAM

Retention time: 3.5 (Asp), 4 (Glu), 8 (Ser), 8.4 (His), 8.8 (Pro), 9.2 (Gly), 9.6 (Arg), 10 (Thr), 11 (Ala), 15 (Tyr), 16 (ammonia), 16.7 (Val), 17.5 (Met), 19.5 (Ile), 20 (Leu), 21.5 (Phe), 22 (Cys), 24.5 (Lys)
Limit of detection: 75-1200 fmole

KEY WORDS

derivatization

REFERENCE

Iwaki,K.; Nimura,N.; Hiraga,Y.; Kinoshita,T.; Takeda,K.; Ogura,H. Amino acid analysis by reversed-phase high-performance liquid chromatography. Automatic pre-column derivatization with activated carbamate reagent, *J.Chromatogr.*, **1987**, *407*, 273–279.

SAMPLE

Matrix: solutions
Sample preparation: Mix 10 μL of an amino acid solution in water with 30 μL buffer, add 20 μL 50 mg/mL sodium 1-thio-β-D-glucose, add 20 μL 40 mg/mL o-phthalaldehyde in MeOH, stir thoroughly for 1 min, add 120 μL 50 mM pH 6.05 sodium acetate, inject a 10 μL aliquot. (Prepare buffer by dissolving 500 mg boric acid in 19 mL water and adjusting the pH to 10.40 with KOH solution (45 g KOH in 100 mL water).)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8
Mobile phase: Gradient. A was 50 mM sodium acetate adjusted to pH 6.05 with acetic acid. B was MeOH:100 mM pH 7.60 sodium acetate 90:10. A:B 100:0 for 8 min, to 45:55 over 47 min, wash with 0:100 for 5 min.
Flow rate: 1.2
Injection volume: 10
Detector: F ex 360 em 420 (cut-off filter)

CHROMATOGRAM

Retention time: 6.4 (L-Asp), 5.2 (D-Asp), 14.4 (L-Glu), 15.6 (D-Glu), 18.2 (L-Ser), 20.1 (D-Ser), 25.0 (L-Thr), 28.1 (D-Thr), 29.0 (L-Arg), 30.3 (D-Arg), 31.6 (L-Ala), 34.0 (D-Ala), 37.0 (L-Tyr), 37.3 (D-Tyr), 48.0 (L-Val), 46.4 (D-Val), 46.1 (L-norvaline), 47.2 (D-norvaline), 47.9 (L-Trp), 48.7 (D-Trp), 51.5 (L-Phe), 50.0 (D-Phe), 53.9 (L-Ileu), 52.8 (D-Ileu), 54.0 (L-Leu), 54.9 (D-Leu), 54.3 (L-norleucine), 55.2 (D-norleucine)

Limit of detection: <1 pmole

KEY WORDS
derivatization; chiral

REFERENCE
Jegorov,A.; Triska,J.; Trnka,T.; Cerny,M. Separation of α-amino acid enantiomers by reversed-phase high-performance liquid chromatography after derivatization with *o*-phthaldialdehyde and a sodium salt of 1-thio-β-D-glucose, *J.Chromatogr.*, **1988**, *434*, 417–422.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate 10 μL of a 2.5 mM solution in 100 mM HCl to dryness under reduced pressure, reconstitute with 100 μL MeCN:pyridine:triethylamine:water 50:25:10:15, evaporate to dryness, reconstitute with 100 μL MeCN:pyridine:triethylamine:water 50:25:10:15, add 5 μL phenylisothiocyanate, mix, let stand at room temperature for 5 min, evaporate to dryness, reconstitute with 250 μL MeCN:water 20:70, inject an aliquot.

HPLC VARIABLES
Guard column: ODS-5 (Altex)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 115 mM pH 6.0 ammonium acetate. B was 230 mM pH 6.0 ammonium acetate in MeCN:MeOH:water 44:10:46. A:B from 100:0 to 85:15 over 15 min, to 50:50 over 15 min, to 100:0 over 4 min, maintain at 100:0 for 3 min, return to initial conditions over 13 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9 (Glu), 11 (Asp), 12.5 (Ser), 16.3 (Hyp), 17.3 (Gly), 17.7 (Gln), 18.5 (Asn), 22 (His), 23 (Thr), 23.6 (Ala), 24.6 (Arg), 24.8 (Pro), 32.5 (Tyr), 33.8 (Val), 35 (Met), 35.8 (Cys), 37.4 (Ile), 37.6 (Leu), 38.6 (Phe), 39 (Trp), 39.4 (Lys)
Limit of detection: 50-500 pmole

KEY WORDS
derivatization; comparison with other derivatization procedures

REFERENCE
McClung,G.; Frankenberger,W.T.,Jr. Comparison of reverse-phase high-performance liquid chromatographic methods for precolumn-derivatized amino acids, *J.Liq.Chromatogr.*, **1988**, *11*, 613–646.

SAMPLE
Matrix: solutions
Sample preparation: 2 mL 1 mM Amino acids in 40 mM pH 9.5 lithium carbonate buffer + 1 mL 5.56 mM dansyl chloride in MeCN, shake gently for 2 min, let stand at room temperature for 35 min, add 100 μL 2% ethylamine, inject a 20 μL aliquot. (Purify MeCN by distillation from dansyl chloride.)

HPLC VARIABLES
Guard column: ODS-5 (Altex)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:30 mM pH 7.6 sodium phosphate buffer 10:90 for 0.1 min, to 45:55 over 22.9 min, return to initial conditions over 7 min.
Flow rate: 2
Injection volume: 20
Detector: UV 250

CHROMATOGRAM

Retention time: 9.5 (Asp), 10.5 (Glu), 17 (Asn, Hyp), 18.5 (Ser), 14.8 (Thr), 15.2 (Gly), 15.5 (Ala), 16 (Pro), 17 (Val), 18 (Met), 18.5 (Leu, Ile), 19 (Phe), 19.3 (Trp), 19.5 (Cys), 24 (Lys), 26 (Thr)

Limit of detection: 50-500 pmole

KEY WORDS

derivatization; comparison with other derivatization procedures; protect from light

REFERENCE

McClung,G.; Frankenberger,W.T.,Jr. Comparison of reverse-phase high-performance liquid chromatographic methods for precolumn-derivatized amino acids, *J.Liq.Chromatogr.*, **1988**, *11*, 613–646.

SAMPLE

Matrix: solutions

Sample preparation: 35 μL Solution of amino acids in water + 35 μL 3.3 mg/mL o-phthalaldehyde in MeOH:water 50:50 + 35 μL 4 mg/mL N-acetyl-L-cysteine in MeOH:water 50:50 + 175 μL 400 mM pH 9.4 potassium borate buffer, mix well, let stand at room temperature for at least 2 min, neutralize with 140 μL 1 M pH 3.5 sodium phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil-120-C18 or 125 × 4 3 μm Nucleosil-120-C18

Mobile phase: MeOH:buffer 40:60 (Buffer was 2.5 mM copper(II) acetate containing 5 mM L-proline adjusted to pH 6.0 with ammonium acetate.)

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: F ex 338 (bandpass filter) em 415 (longpass filter)

CHROMATOGRAM

Retention time: k' 1.8 (L-valine), k' 2.4 (D-valine), k' 2.8 (L-leucine), k' 3.2 (D-leucine)

OTHER SUBSTANCES

Also analyzed: other amino acids, α-alkylamino acids, amino acid amides

KEY WORDS

derivatization; chiral

REFERENCE

Duchateau,A.; Crombach,M.; Kamphuis,J.; Boesten,W.H.J.; Schoemaker,H.E.; Meojer,E.M. Determination of the enantiomers of α-H-α-amino acids, α-alkyl-α-amino acids and the corresponding acid amides by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *471*, 263–270.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve amino acids in 20 μL 50 mM pH 9.0 sodium bicarbonate, add 40 μL 4 mM dabsyl chloride in MeCN, heat at 70° for 10 min, dry under vacuum, dissolve the residue in EtOH:water 70:30 (J. Chromatogr. 1985, 349 77), inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18

Column: 150 × 4.6 3 μm Supelcosil LC-18

Mobile phase: Gradient. A was 25 mM pH 6.8 KH_2PO_4. B was MeCN:isopropanol 75:25. A:B 80:20 for 1 min, to 77:23 over 3 min, maintain at 77:23 for 5 min, to 73:27 over 1 min,

maintain at 73:27 for 4 min, to 65:35 over 5 min, to 40:60 over 6 min, to 70:30 over 1 min, maintain at 30:70 for 3 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 2
Injection volume: 5
Detector: UV 436

CHROMATOGRAM

Retention time: 4 (aspartic acid), 4.5 (cysteamine), 5 (glutamic acid), 5.7 (carboxymethylcysteine), 6 (S-sulfocysteine), 8.5 (asparagine), 9.5 (glutamine), 10 (serine), 10.7 (threonine), 11.4 (glycine), 12 (alanine), 12.5 (arginine), 13.5 (taurine), 14 (proline), 14.5 (valine), 16.4 (methionine), 17.2 (isoleucine), 17.7 (leucine), 18.2 (tryptophan), 18.5 (norleucine), 19 (phenylalanine), 20 (ammonia), 20.5 (cystine), 22.4 (OH-Lys), 23 (lysine), 23.5 (histidine), 24.3 (tyrosine)

KEY WORDS

derivatization

REFERENCE

Stocchi,V.; Piccoli,G.; Magnani,M.; Palma,F.; Biagiarelli,B.; Cucchiarini,L. Reversed-phase high-performance liquid chromatography separation of dimethylaminoazobenzene sulfonyl- and dimethylaminoazobenzene thiohydantoin-amino acid derivatives for amino acid analysis and microsequencing studies at the picomole level, *Anal.Biochem.*, **1989**, *178*, 107–117.

SAMPLE

Matrix: solutions
Sample preparation: Add a 10-fold excess of 1,1-diphenylborinic acid to a solution of amino acids in 1 M acetic acid in isopropanol:water 50:50, heat at 65-70° for 15-20 min, dry under reduced pressure, reconstitute with mobile phase, inject an aliquot. (Prepare 1,1-diphenylborinic acid as follows. Stir diphenylborinic acid ethanolamine ester in 1 M HCl under nitrogen for 30 min, extract with dichloromethane. Wash the organic layer twice with water, wash with brine, dry over anhydrous sodium sulfate, evaporate under reduced pressure, take up the oil in warm hexane, cool, collect and wash the crystals, recrystallize from hexane to obtain 1,1-diphenylborinic acid (mp 128-130°) (Biomed. Mass Spec. 1984, 11, 611).)

HPLC VARIABLES

Column: 250 × 4 Zorbax PTH
Mobile phase: MeCN:THF:6 mM pH 3.30 phosphoric acid 18:16:66
Detector: UV 254

CHROMATOGRAM

Retention time: 8.56 (phosphorylated serine), 10.78 (phosphorylated threonine), 11.33 (His), 13.77 (Asn), 14.22 (Gln), 14.63 (Gla), 15.53 (Ser), 15.63 (phosphorylated tyrosine), 16.13 (Asp), 16.63 (Gly), 16.94 (Thr), 17.12 (Glu), 18.30 (N-methylglycine), 18.75 (Ala), 19.12 (Pro), 20.70 (N,N-dimethylglycine), 21.97 (biocytin), 25.96 (Cys), 27.54 (gamma-methylglutamic acid), 28.49 (Val), 29.40 (glutathione), 29.99 (Tyr), 30.76 (Lys), 32.43 (Arg), 33.16 (diphthine), 34.52 (Met), 37.64 (Ile), 40.18 (Leu), 45.3 (Phe), 48.43 (Trp), 75.97 (S-(p-nitrobenzyl)glutathione), 89.69 (2,6-diaminopimelic acid), 90.60 (oxidized glutathione), 102.51 (glutamyllysine)

OTHER SUBSTANCES

Noninterfering: amines, peptides

KEY WORDS

derivatization

REFERENCE

Strang,C.J.; Henson,E.; Okamoto,Y.; Paz,M.A.; Gallop,P.M. Separation and determination of α-amino acids by boroxazolidone formation, *Anal.Biochem.*, **1989**, *178*, 276–286.

SAMPLE

Matrix: solutions

Sample preparation: Mix 500 μL of a 10 μM solution of amino acids in 100 mM pH 9.3 borax buffer containing 1 mM disodium EDTA with 500 μL 20 mM DBD-F in MeCN, heat at 50° for 30 min, cool in ice, inject a 1-10 μL aliquot. (Synthesis of DBD-F is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 60F_{254} tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR).)

HPLC VARIABLES

Guard column: 20 × 3.9 37-50 μm Bondapak C18/Corasil

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 90:10 to 30:70 over 1 h.

Column temperature: 40

Flow rate: 1

Injection volume: 1-10

Detector: F ex 450 em 590

CHROMATOGRAM

Retention time: 10 (hydroxyproline), 12 (glycine), 15 (alanine), 19 (proline), 23 (valine), 27 (isoleucine), 28 (phenylalanine), 30 (lysine), 35 (tyrosine)

Limit of detection: 0.11-0.79 pmole

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Suzuki,T.; Saito,Y.; Uzu,S.; Imai,K. Evaluation of benzofurazan derivatives as fluorigenic reagents for thiols and amines using high-performance liquid chromatography, *Analyst*, **1989**, *114*, 1233–1240.

SAMPLE
Matrix: solutions
Sample preparation: Dry an aqueous solution of amino acids into a tube, add 10 μL reagent, vortex vigorously, let stand for 10 min, add 40 μL water, wash with 50 μL toluene, inject a 1-10 μL aliquot of he aqueous layer. (Prepare reagent by mixing 490 μL 50' mM 4-nitrophenyl isothiocyanate in MeCN, 50 μL 10% triethylamine in MeCN, and 50 μL water. Purify commercial 4-nitrophenyl isothiocyanate by sublimation at 96° and 13 mm Hg to give a pale yellow powder (mp 106.5°).)

HPLC VARIABLES
Column: 300 × 3.9 Pico-Tag free amino acid analysis column (Waters)
Mobile phase: Gradient. A was MeCN:140 mM sodium acetate:triethylamine 6:94:0.05, pH adjusted to 6.4 with acetic acid. B was MeCN:water 60:40. A:B from 85:15 to 40:60 over 20 min, to 0:100 (step gradient), maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 12 min.
Column temperature: 46
Flow rate: 1
Injection volume: 1-10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.3 (Asp), 3.5 (Glu), 7.2 (Ser), 8 (Gly), 8.3 (His), 9 (Arg), 9.8 (Thr), 10 (Ala), 10.7 (Pro), 12.2 (Tyr), 13.3 (Val), 14 (Met), 15.5 (Ile), 15.7 (Leu), 16 (cystine), 16.8 (Phe), 21.7 (Lys)
Limit of detection: 0.5-1 pmole

KEY WORDS
derivatization

REFERENCE
Cohen,S.A. Analysis of amino acids by liquid chromatography after precolumn derivatization with 4-nitrophenylisothiocyanate, *J.Chromatogr.*, **1990**, *512*, 283–290.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was 50 mM pH 7.0 sodium phosphate buffer. B was MeOH: THF:water 50:20:30. A:B from 25:75 to 0:100 over 75 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM

Retention time: 15 (aspartic acid), 20 (glutamic acid), 27.5 (asparagine), 30 (glutamine), 31.5 (serine), 32.5 (homoserine), 35.5 (glycine), 40 (taurine), 42 (alanine), 44 (β-alanine), 52 (norvaline), 54 (valine), 60 (leucine)

KEY WORDS

derivatization

REFERENCE

Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE

Matrix: solutions

Sample preparation: Mix 5 μL of a 0.05-10 mM solution with 20 μL 100 mM NaOH and 150 μL 5 mM 4-(N-phthalimidyl)benzenesulfonyl chloride in acetone, heat at 50° for 15 min, dilute 10-fold with mobile phase, inject a 20 μL aliquot. (Synthesis of 4-(N-phthalimidyl)benzenesulfonyl chloride (Phisyl-Cl) is as follows. Mix 2.68 g o-phthalaldehyde in 100 mL diethyl ether with 1.86 g aniline in 20 mL diethyl ether, stir at room temperature overnight, filter. Wash the solid with diethyl ether and recrystallize it from MeOH to yield N-phenylphthalimidine. Drop 6.6 g chlorosulfonic acid onto 2.09 g of crystals of N-phenylphthalimidine in an ice bath with vigorous stirring over 20 min, heat at 60° for 2 h, add 30 g crushed ice, recrystallize the precipitate from benzene to obtain 4-(N-phthalimidyl)benzenesulfonyl chloride as fine colorless needles (mp 186-187°) (Caution! Benzene is a carcinogen!).)

HPLC VARIABLES

Guard column: 25 × 4.6 5 μm ODS (Yamamura, Japan)

Column: 250 × 4.6 5 μm YMC AM-303 ODS (Yamamura)

Mobile phase: Gradient. A was MeCN:30 mM pH 6.5 Tris buffer 10:90. B was MeCN:30 mM pH 6.5 Tris buffer 75:25. A:B 100:0 for 8 min, 90:10 for 12 min, 80:20 for 18 min, 70:30 for 10 min, 55:45 for 5 min, 40:60 for 7 min, 0:100 for 5 min, re-equilibrate at initial conditions for 15 min (step gradient).

Flow rate: 0.6

Injection volume: 20

Detector: F ex 295 em 425

CHROMATOGRAM

Retention time: 18.5 (cysteine), 19 (aspartic acid), 20 (glutamic acid), 30 (hydroxyproline), 32.5 (asparagine), 35 (serine), 36 (methionine), 36.5 (threonine), 38 (glycine), 39 (alanine), 43 (proline), 47 (valine), 51 (isoleucine), 52.5 (leucine), 55 (phenylalanine), 56.5 (cystine), 59.8 (ornithine), 60.2 (lysine), 61 (histidine), 63 (tyrosine)

Limit of detection: <0.2 pmole

KEY WORDS

derivatization

REFERENCE

Tsuruta,Y.; Date,Y.; Kohashi,K. Phthalimidylbenzenesulfonyl chlorides as fluorescence labeling reagents for amino acids in high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *502*, 178–183.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 200 ppm solution in MeCN:50 mM NaOH 80:20 with 30 mg reagent, after 2 min elute with 500 μL MeCN, add 500 μL water to the eluate,

mix, inject a 20 μL aliquot. (The reagent was dinitrobenzoylbenzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb CN 100
Mobile phase: MeCN:water:trifluoroacetic acid 20:80:0.1
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: 2 (valine), 2.2 (methionine), 3.3 (phenylalanine), 4 (tryptophan)

KEY WORDS

derivatization

REFERENCE

Bourque,A.J.; Krull,I.S. Solid-phase reagent containing the 3,5-dinitrophenyl tag for the improved derivatization of chiral and achiral amines, amino alcohols and amino acids in high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1991**, *537*, 123–152.

SAMPLE

Matrix: solutions
Sample preparation: Mix 200 μL of a 10 mM solution of amino acid in 100 mM sodium bicarbonate with 200 μL 10 mM Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent) in acetone (freshly prepared), heat at 40° with frequent mixing for 1 h, cool, add 100 μL 200 mM HCl, degas, filter, inject an aliquot.

HPLC VARIABLES

Column: 100 × 8 10 μm μBondapak
Mobile phase: Gradient. X was MeCN containing 0.1% trifluoroacetic acid. Y was water containing 0.1% trifluoroacetic acid. X:Y from 10:90 to 60:40 over 50 min (mobile phase A) or from 5:95 to 20:80 over 70 min (Mobile Phase B) or isocratic MeCN:20 mM pH 4.0 sodium acetate buffer 8:92
Flow rate: 2
Detector: UV 340

CHROMATOGRAM

Retention time: k' 2.96 (L-His) (Mobile Phase A), k' 1.80 (D-His) (Mobile Phase A), k' 4.18 (L-Asp) (Mobile Phase A), k' 4.66 (D-Asp) (Mobile Phase A), k' 4.80 (L-Thr) (Mobile Phase A), k' 6.11 (D-Thr) (Mobile Phase A), k' 5.70 (L-Glu) (Mobile Phase A), k' 6.37 (D-Glu) (Mobile Phase A), k' 6.73 (L-Ala) (Mobile Phase A), k' 7.72 (D-Ala) (Mobile Phase A), k' 7.55 (L-Val) (Mobile Phase A), k' 9.08 (D-Val) (Mobile Phase A), k' 7.95 (L-Leu-NH2) (Mobile Phase A), k' 9.73 (D-Leu-NH2) (Mobile Phase A), k' 8.14 (L-Tyr) (Mobile Phase A), k'

9.05 (D-Tyr) (Mobile Phase A), k' 8.12 (L-Trp-NH2) (Mobile Phase A), k' 9.40 (D-Trp-NH2) (Mobile Phase A), k' 8.17 (L-Met) (Mobile Phase A), k' 9.85 (D-Met) (Mobile Phase A), k' 8.92 (L-Phe-NH2) (Mobile Phase A), k' 10.57 (D-Phe-NH2) (Mobile Phase A), k' 9.02 (L-Leu) (Mobile Phase A), k' 10.65 (D-Leu) (Mobile Phase A), k' 9.51 (L-Ile) (Mobile Phase A), k' 11.45 (D-Ile) (Mobile Phase A), k' 9.83 (L-Trp) (Mobile Phase A), k' 11.25 (D-Trp) (Mobile Phase A), k' 9.96 (L-Nle) (Mobile Phase A), k' 11.87 (D-Nle) (Mobile Phase A), k' 10.18 (L-allylglycine) (Mobile Phase A), k' 11.69 (D-allylglycine) (Mobile Phase A), k' 10.44 (L-Cys (tert-butyl)) (Mobile Phase A), k' 12.01 (D-Cys (tert-butyl)) (Mobile Phase A), k' 10.80 (L-Cystine) (Mobile Phase A), k' 11.47 (D-Cystine) (Mobile Phase A), k' 11.04 (L-Phe) (Mobile Phase A), k' 12.40 (D-Phe) (Mobile Phase A), k' 11.89 (L-Lys (bis derivative)) (Mobile Phase A), k' 12.58 (D-Lys (bis derivative)) (Mobile Phase A), k' 13.54 (L-His (triphenylmethyl)) (Mobile Phase A), k' 13.89 (D-His (triphenylmethyl)) (Mobile Phase A), k' 16.29 (L-Cys (triphenylmethyl)) (Mobile Phase A), k' 17.21 (D-Cys (triphenylmethyl)) (Mobile Phase A), k' 17.80 (L-Ser) (Mobile Phase B), k' 16.63 (D-Ser) (Mobile Phase B), k' 20.48 (L-Cys (S-acetamidomethyl)) (Mobile Phase B), k' 22.67 (D-Cys (S-acetamidomethyl)) (Mobile Phase B), k' 25.08 (L-Pro) (Mobile Phase B), k' 26.68 (D-Pro) (Mobile Phase B), k' 26.2 (L-Asn) (Mobile Phase C), k' 44.7 (D-Asn) (Mobile Phase C), k' 26.6 (L-Gln) (Mobile Phase C), k' 45.7 (D-Gln) (Mobile Phase C), k' 51.7 (L-Arg) (Mobile Phase C), k' 103.3 (D-Arg) (Mobile Phase C)

KEY WORDS
chiral; derivatization

REFERENCE
Adamson,J.G.; Hoang,T.; Crivici,A.; Lajoie,G.A. Use of Marfey's reagent to quantitate racemization upon anchoring of amino acids to solid supports for peptide synthesis, *Anal.Biochem.*, **1992**, *202*, 210–214.

SAMPLE
Matrix: solutions
Sample preparation: Mix 70 μL of an aqueous amino acid solution with 300 μL phthalaldehyde solution and 30 μL thiol solution for 2 min, inject an aliquot. (Prepare the phthalaldehyde solution by dissolving 60 mg o-phthalaldehyde in 3 mL MeOH and 15 mL 400 mM pH 9.4 sodium borate buffer. Prepare the thiol solution by dissolving 6.5 mg D-S-acetyl-3-mercapto-2-methylpropionic acid (Novabiochem) in 1 mL 1 M NaOH, stir at room temperature for 10 min, adjust the pH to 7.0 with phosphoric acid. The solution contains D-3-mercapto-2-methylpropionic acid.)

HPLC VARIABLES
Column: 250 × 4 3 μm Nucleosil-120-C18
Mobile phase: Gradient. MeCN:50 mM pH 6.0 sodium acetate buffer 0:100 for 10 min, to 62.5:37.5 (?) over 100 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 338 em 415 (long-pass filter)

CHROMATOGRAM
Retention time: 21 (D-Asp), 25 (L-Asp), 28.5 (D-Glu), 32 (L-Glu), 33 (D-Asn), 34 (D-Ser), 36 (L-Asn), 37 (L-Ser), 37.5 (D-Gln), 41 (D-Thr), 42 (L-Gln), 42.5 (Gly), 43 (D-His), 43.5 (L-His), 45 (D-Arg), 46 (L-Thr), 48 (D-Ala), 49 (L-Arg), 51.5 (L-Ala), 54 (D-Tyr), 57 (L-Tyr), 61 (D-Val), 64 (D-Met), 68 (L-Met), 69 (D-Ile, L-Val), 70 (D-Trp), 71 (D-Phe), 72 (D-Leu), 74 (L-Trp), 75 (L-Phe), 78.5 (L-Ile), 80 (L-Leu), 91 (D-Lys), 93 (L-Lys)
Limit of detection: 2 pmole

KEY WORDS
derivatization; comparison with other thiols; chiral

REFERENCE

Duchateau,A.L.L.; Knuts,H.; Boesten,J.M.M.; Guns,J.J. Enantioseparation of amino compounds by derivatization with o-phthalaldehyde and D-3-mercapto-2-methylpropionic acid, *J.Chromatogr.*, **1992**, *623*, 237–245.

SAMPLE

Matrix: solutions

Sample preparation: Mix 10 μL of a 100 μM solution in 200 mM pH 9.3 borate buffer containing 4 mM disodium EDTA with 100 μL 50 mM DBD-F in MeCN, heat at 60° for 30 min, add 890 μL MeOH:acetic acid 99:1, inject a 10 μL aliquot. (Synthesis of DBD-F is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products. It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Sumichiral OA 2500(S) ((S)-1-naphthylglycyl-3,5-dinitrophenylamide silica gel) (Sumika Analytical, Osaka)

Mobile phase: MeOH containing 20 mM ammonium acetate

Flow rate: 1

Injection volume: 10

Detector: F ex 450 em 590

CHROMATOGRAM

Retention time: 13 (D-Leu), 15 (L-Leu)

KEY WORDS
derivatization; chiral; comparison with other derivatizing reagents

REFERENCE
Imai,K.; Fukushima,T. Derivatization with fluorogenic benzofurazan reagents of amino acid enantiomers and their separation on a Pirkle type column, *Biomed.Chromatogr.*, **1993**, *7*, 275–276.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL of a 100 μM solution in 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA with 30 μL 50 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole in MeCN, heat at 60° for 2 min, add 960 μL MeOH:acetic acid 99:1, dilute 5 times with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumichiral OA 2500(S) ((S)-1-naphthylglycyl-3,5-dinitrophenylamide silica gel) (Sumika Analytical, Osaka)
Mobile phase: MeOH containing 20 mM ammonium acetate
Flow rate: 1
Injection volume: 10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 18.57 (D-Leu), 20.8 (L-Leu), 25.3 (D-Phe), 30.6 (L-Phe)
Limit of detection: 25-34 fmole

KEY WORDS
derivatization; chiral

REFERENCE
Imai,K.; Fukushima,T.; Uzu,S. Sensitive determination of enantiomers of amino acids derivatized with the fluorogenic reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole, separated on a Pirkle-type column, Sumichiral OA 2500(S), *Biomed.Chromatogr.*, **1993**, *7*, 177–178.

SAMPLE
Matrix: solutions
Sample preparation: 32 μL Amino acid solution in 50 mM pH 9.0 borate buffer + 69 μL 1.96 mM cyanide, mix, add 6 μL 2.25 mM naphthalene dicarboxaldehyde mixed with 13 μL 50 mM pH 9.0 borate buffer, mix, let stand for 30 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 MicroPak SP C18
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 10:90. B was MeCN:MeOH:THF:50 mM pH 6.8 potassium phosphate buffer 55:10:3.5:31.5. A:B from 90:10 to 45:55 over 45 min, to 20:80 over 1 min, maintain at 20:80 for 3 min, return to initial conditions over 2 min, re-equilibrate for 3 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 420 em 490

CHROMATOGRAM
Retention time: 7 (aspartic acid), 10 (glutamic acid), 18 (histidine), 20 (serine), 22 (arginine), 22.5 (glycine), 24 (threonine), 26 (alanine), 29 (tyrosine), 31 (α-aminobutyric acid), 35.5 (valine), 36 (methionine), 40.5 (isoleucine), 41 (phenylalanine), 42 (leucine)
Limit of detection: 200 fmole

KEY WORDS
derivatization; discussion of matrix interference in paper

REFERENCE
Lai,F.; Sheehan,T. Matrix effects in the derivatization of amino acids with naphthalene dicarboxaldehyde, 9-fluorenylmethyl chloroformate and phenylisothiocyanate, *BioTechniques*, **1993**, *14*, 642–649.

SAMPLE
Matrix: solutions
Sample preparation: Mix 250 μL of a solution of amino acids in 50 mM pH 9.3 borate buffer containing 1 mM disodium EDTA with 250 μL 1 mM DIFOX in MeCN, let stand in the dark at room temperature for 1 h, add 500 μL MeCN:1 M HCl 50:50, inject an aliquot. (Prepare DIFOX as follows. Gently reflux 21 g benzoin and 45 g urethane (Caution! Urethane is a carcinogen!) in 300 mL DMF for 6 h, cool, pour into water, filter, recrystallize to give 4,5-diphenyl-2-oxazolone (mp 211°), treat with phosphorus oxychloride to give 2-chloro-4,5-diphenyloxazole (Ber. 1956, 89, 1749). Add 3 g anhydrous KF to 1.62 g 2-chloro-4,5-diphenyloxazole in 60 mL MeCN, add 1.5 g 18-crown-6/MeCN complex, reflux for 24 h, cool, filter. Concentrate the filtrate and add 50 mL hexane, stir for 10 min, filter rapidly, repeat the extraction. Combine the filtrates and concentrate them to give a yellow oil, distil to give DIFOX (2-fluoro-4,5-diphenyloxazole) (bp 130°/0.02 mm Hg) (Analyst 1993, 118, 257). Prepare 18-crown-6/MeCN complex by heating 50 g 18-crown-6 in 125 mL MeCN until a homogeneous solution is obtained (use a calcium sulfate drying tube), stir the solution vigorously as it cools to room temperature, cool in a dry ice/acetone bath, filter rapidly, dry under high vacuum at ≤40° over 2-3 h to give 18-crown-6/MeCN complex (mp 36.5-38°) (Caution! The complex is hygroscopic!) (J. Org. Chem. 1974, 39, 2445). No experimental details are given for the phosphorus oxychloride reaction above but a procedure for the p-N,N-dimethylaminosulfonyl analogue proceeds as follows. Suspend 2 g dried 4,5-bis(p-N,N-dimethylaminosulfonylphenyl)-2-oxazolone in 30 mL phosphorus oxychloride, stir at 0°, add 610 μL triethylamine dropwise, heat at 100° for 7 h, remove the excess phosphorus oxychloride on a rotary evaporator. Dissolve the residue in dichloromethane and wash with cold saturated sodium bicarbonate, dry the organic layer over anhydrous magnesium sulfate, evaporate to dryness, chromatograph on silica gel to give 2-chloro-4,5-bis(p-N,N-dimethylaminosulfonylphenyl)oxazole (Analyst 1993, 118, 257).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-8 (Supelco)
Mobile phase: Gradient. A was MeCN:50 mM pH 7.0 phosphate buffer 25:75. B was MeCN: 50 mM pH 7.0 phosphate buffer 50:50. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 30 min.
Flow rate: 1
Detector: F ex 320 em 420

CHROMATOGRAM
Retention time: 6 (aspartic acid), 6.5 (glutamic acid), 9.5 (hydroxyproline), 13.5 (arginine), 15 (proline), 15.5 (glycine), 16 (alanine), 17.5 (tyrosine), 20 (valine), 20.5 (methionine), 21.5 (leucine, isoleucine), 23.5 (tryptophan, phenylalanine), 35 (lysine)
Limit of detection: 19-64 fmole

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Chokshi,H.P.; Givens,R.S.; Carlson,R.G.; Lunte,S.M.; Kuwana,T. Fluorescence and chemiluminescence detection of oxazole-labelled amines and thiols, *Biomed.Chromatogr.*, **1993**, *7*, 208–216.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL 4 mM amino acids in water with 100 μL buffer, 50 μL 50 mg/mL 1-thio-β-D-galactopyranose sodium salt in water, and 50 μL 40 mg/mL o-

phthalaldehyde in MeOH, stir thoroughly for 1 min, inject a 10 μL aliquot. (Buffer was 500 mg boric acid in 19 mL water, pH adjusted to 9.30 with 45% KOH.)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8
Mobile phase: Gradient. A was 50 mM sodium acetate adjusted to pH 6.10 with acetic acid. B was MeOH:100 mM pH 7.60 sodium acetate buffer 90:10. A:B 100:0 for 8 min, to 45:55 over 47 min, to 0:100 over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 10
Detector: F ex 360 em 420

CHROMATOGRAM

Retention time: 4.6 (L-Asp, D-Asp), 8.9 (L-Glu, D-Glu), 12.1 (L-Ser), 15.2 (D-Ser), 20.7 (L-Tre), 24.2 (D-Tre), 27.7 (L-Ala), 29.9 (L-Arg), 30.1 (D-Ala), 30.3 (D-Arg), 37.1 (L-Tyr), 37.8 (D-Tyr), 43.0 (D-Val), 44.9 (L-norvaline), 45.1 (L-Trp), 45.4 (L-Val), 45.9 (D-norvaline), 46.8 (D-Trp), 47.6 (L-Phe), 49.6 (D-Phe), 51.8 (L-Leu), 51.8 (D-Ile), 52.2 (L-norleucine), 52.2 (L-Ile), 53.1 (D-norleucine), 53.1 (D-Leu), 54.2 (D-Lys), 55.5 (L-Lys)
Limit of detection: <10 pmole

KEY WORDS

derivatization; chiral

REFERENCE

Jegorov,A.; Triska,J.; Trnka,T. 1-Thio-β-D-galactose as a chiral derivatization agent for the resolution of D,L-amino acid enantiomers, *J.Chromatogr.A*, **1994**, *673*, 286–290.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate a solution of amino acids in 10 mM HCl under a stream of nitrogen at 50°, add 50 μL MeCN, evaporate under a stream of nitrogen, add 50 μL MeCN:MeOH:triethylamine 10:5:2, sonicate for 1 min, add 3 μL butyl isothiocyanate, cap the vial with a septum, heat at 40° for 30 min. Pass nitrogen into the vial with one needle and apply a vacuum with another needle until solvent is removed (ca. 15 min), add 100 μL MeCN, remove MeCN in a similar fashion. Dissolve the residue in 500 μL 200 mM ammonium acetate, filter (0.20 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 50 mM ammonium acetate adjusted to pH 6.7 with phosphoric acid. B was MeCN:50 mM ammonium acetate adjusted to pH 6.7 with phosphoric acid 50:50. C was MeCN:water 70:30. A:B:C from 100:0:0 to 85:15:0 over 8 min, to 70:20:10 over 6 min, to 60:20:20 over 6 min, to 20:0:80 over 5 min, to 0:0:100 over 5 min, maintain at 0:0:100 for 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 250

CHROMATOGRAM

Retention time: 8.05 (Asp), 9.00 (Glu), 11.27 (Hyp), 13.75 (Asn), 13.75 (Ser), 14.39 (Gly), 14.63 (Gln), 15.71 (His), 16.13 (Thr), 16.29 (Ala), 16.45 (Arg), 16.84 (Pro), 17.76 (Cyt), 20.27 (Tyr), 20.51 (Val), 21.73 (Met), 23.29 (Ile), 23.67 (Leu), 25.18 (Phe), 25.53 (Trp), 26.16 (Lys), 26.84 (Cys)
Limit of quantitation: 0.5 nmole

KEY WORDS
Asp and Ser not resolved; derivatization

REFERENCE
Woo,K.-L.; Lee,S.-H. Determination of protein amino acids as butylthiocarbamyl derivatives by reversed-phase high-performance liquid chromatography with precolumn derivatization and UV detection, *J.Chromatogr.A*, **1994**, *667*, 105–111.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of an amino acid solution with 100 μL 500 mM pH 9.0 sodium borate buffer and 250 μL 5 mM 4-phenylazobenzyloxycarbonyl chloride in MeCN, let stand for 5 min, add 250 μL 40 mM 1-aminoadamantane in acetone:water 75:25, mix, let stand for 5 min. Remove an 80 μL aliquot and add it to 320 μL MeCN:500 mM pH 4.0 sodium acetate buffer 50:50, inject a 20 μL aliquot. (4-Phenylazobenzyloxycarbonyl chloride can be purchased from Bachem, Bubendorf, Switzerland. Synthesis is as follows. Dissolve 10 g 4-nitrobenzylalcohol in 100 mL MeOH, add 1 mL triethylamine, add 2 g Raney nickel, hydrogenate at room temperature and atmospheric pressure, filter. Evaporate the filtrate to dryness, add benzene (Caution! Benzene is a carcinogen!), evaporate to dryness to remove water, repeat this step to obtain 4-aminobenzyl alcohol as crystals. Mix 7.7 g 4-aminobenzyl alcohol with a solution of 7.4 g nitrosobenzene in 40 mL acetic acid with stirring at 0°, after 3 h filter, wash the solid with dilute acetic acid to obtain 4-phenylazobenzylalcohol (mp 142.5-143°). Dilute the filtrate with a lot of water, filter, extract with hot carbon tetrachloride, crystallize from carbon tetrachloride to obtain more p-phenylazobenzyl alcohol. Dissolve 10.9 g phosgene in 40 mL dioxane, add 5 g p-phenylazobenzylalcohol at 0°, stir at 0° for 15 min, let stand at room temperature for 3 h, filter, evaporate the filtrate to dryness under reduced pressure. Recrystallize the residue from petroleum ether to give 4-phenylazobenzyloxycarbonyl (mp 82-83°) (Helv. Chim. Acta 1958, 41, 491).)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: Gradient. MeCN:100 mM pH 7.0 sodium acetate from 22:78 to 50:50 over 40 min, to 80:20 over 5 min, to 100:0 over 1 min, maintain at 100:0 for 9 min, re-equilibrate at initial conditions for 12 min.
Column temperature: 45
Flow rate: 1.25
Injection volume: 20
Detector: UV 320

CHROMATOGRAM
Retention time: 7 (Asp), 8 (Glu), 14 (Ser), 15.5 (Gly), 16 (Thr), 16.5 (Pro, Arg), 17 (Ala), 19 (Tyr (mono-derivative)), 22 (Val), 22.5 (Met), 25 (Ile), 25.5 (Leu), 26.5 (Phe), 31.5 (cystine), 42.5 (His), 43 (Lys), 47 (Tyr (bis-derivative))
Limit of detection: 1-10 pmole

KEY WORDS
derivatization; comparison with other derivatizing reagents

REFERENCE
Brückner,H.; Lüpke,M. Use of chromogenic and fluorescent oxycarbonyl chlorides as reagents for amino acid analysis by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *697*, 295–307.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of an amino acid solution with 100 μL 400 mM pH 8.0 sodium borate buffer and 100 μL 3 mM 9-fluorenylmethyl chloroformate in acetone

(MeCN if UV detection is used), let stand for 2 min, add 100 μL 40 mM 1-aminoadamantane in acetone:water 75:25, let stand for 2 min. Remove an 20 μL aliquot and add it to 380 μL MeCN:500 mM pH 4.0 sodium acetate buffer 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-8
Column: 250 × 4 4 μm Superspher 60 RP-8
Mobile phase: Gradient. A was THF:DMF:100 mM pH 4.6 sodium acetate buffer 5:5:90. B was MeCN. A:B from 93:7 to 85:15 over 10 min, to 50:50 over 25 min, to 0:100 over 5 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 10 min. (Reflux DMF in the presence of ninhydrin then distil.)
Column temperature: 45
Flow rate: 1.25
Injection volume: 20
Detector: F ex 263 em 313

CHROMATOGRAM

Retention time: 8 (CyA), 15.2 (Asn), 16 (Gln), 17 (Asp), 17.5 (Ser), 18.5 (Glu), 19.5 (Thr), 20 (Arg), 20.5 (Gly), 21 (homo-Arg), 23 (Ala), 24 (Tyr, mono-derivative), 25 (Pro), 26 (Met), 28 (Val, GABA), 29 (Phe), 30.8 (Ile), 32 (Leu), 32.5 (Hyl), 34 (His), 35 (Orn), 36.5 (Lys), 38 (Tyr, bis-derivative)
Limit of detection: 5-150 fmole

KEY WORDS

derivatization; comparison with other derivatizing reagents

REFERENCE

Brückner,H.; Lüpke,M. Use of chromogenic and fluorescent oxycarbonyl chlorides as reagents for amino acid analysis by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *697*, 295–307.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL Amino acid solution in 500 mM pH 8 sodium borate buffer + 50 μL 20 mM norvaline in 500 mM pH 8 sodium borate buffer + 200 μL 30 mM 9-fluorenylmethyl chloroformate in dry acetone, shake, allow to stand at room temperature for 10 min, add 200 μL 25 mM 1-aminoamantadine in MeOH, let stand for 2 min, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Aminotag (Varian)
Mobile phase: Gradient. A was MeCN:isopropanol 90:10. B was THF:50 mM sodium acetate buffer 4:96, adjusted to pH 4.03 with glacial acetic acid. A:B from 20:80 to 25:75 over 25 min, to 30:70 over 10 min, to 40:60 over 10 min, to 50:50 over 10 min, to 80:20 over 15 min, to 100:0 over 5 min, re-equilibrate at initial conditions for 15 min.
Column temperature: 32
Flow rate: 1.5
Injection volume: 20
Detector: UV 265 or F ex 265 em 340

CHROMATOGRAM

Retention time: 12 (arginine), 15 (taurine), 16 (serine), 17 (aspartic acid), 20 (glutamic acid), 21 (threonine), 22 (glycine), 31 (proline), 37 (alanine), 41.5 (methionine), 43.5 (valine), 48 (phenylalanine), 48.5 (tryptophan), 49 (isoleucine), 50 (leucine), 59 (histidine), 60.5 (lysine), 64 (tyrosine)
Internal standard: norvaline (45)

KEY WORDS

derivatization

REFERENCE
Carratù,B.; Boniglia,C.; Bellomonte,G. Optimization of the determination of amino acids in parenteral solutions by high-performance liquid chromatography with precolumn derivatization using 9-fluorenylmethyl chloroformate, *J.Chromatogr.A*, **1995**, *708*, 203–208.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL 20 mM Amino acid (10 mM for Lys) in MeCN:water 50:50 + 2 μL triethylamine + 20 μL 40 mg/mL O-tetraacetyl-β-D-glucopyranosyl isothiocyanate in MeCN, let stand at room temperature for 30 min, add 50 μL 1 M HCl, add 830 μL MeCN:water 25:75, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 7 μm Hypercarb S
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN:water 90:10. A:B 70:30 for 15 min, to 64:36 over 10 min, maintain at 64:36 for 3 min, to 61:39 over 5 min, maintain at 61:39 for 2 min, to 55:45 over 10 min, maintain at 55:45 for 5 min, to 0:100 over 33 min, maintain at 0:100 for 10 min, return to initial conditions over 3 min.
Flow rate: 1.1
Injection volume: 5-20
Detector: UV 250

CHROMATOGRAM
Retention time: k' 7.15 (D-His), k' 4.38 (L-His), k' 10.54 (D-Arg), k' 8.92 (L-Arg), k' 11.89 (D-Ser), k' 11.60 (L-Ser), k' 12.43 (D-Pro), k' 12.78 (L-Pro), k' 16.67 (D-Thr), k' 13.71 (L-Thr), k' 17.07 (D-Ala), k' 14.49 (L-Ala), k' 17.97 (D-Asp), k' 16.03 (L-Asp), k' 17.97 (D-Glu), k' 16.03 (L-Glu), k' 25.52 (D-Val), k' 21.69 (L-Val), k' 28.02 (D-Leu), k' 25.17 (L-Leu), k' 29.9 (D-Ile), k' 27.41 (L-Ile), k' 41.0 (D-Phe), k' 34.27 (L-Phe), k' 37.17 (D-Lys), k' 36.65 (L-Lys), k' 45.04 (D-Tyr), k' 34.67 (L-Tyr)

KEY WORDS
chiral; derivatization

REFERENCE
Chan,W.C.; Micklewright,R.; Barrett,D.A. Porous graphitic carbon for the chromatographic separation of O-tetraacetyl-β-D-glucopyranosyl isothiocyanate-derivatised amino acid enantiomers, *J.Chromatogr.A*, **1995**, *697*, 213–217.

SAMPLE
Matrix: solutions
Sample preparation: Shake a slurry of 100 μmoles amino acid, 100 μmole 3,5-dinitrobenzoyl chloride, and 300 μmoles propylene oxide (Caution! Propylene oxide is a carcinogen!) in 5 mL dry THF was shaken at room temperature for 7 days, filter, evaporate to dryness under reduced pressure, dissolve the residue in 30 mL MeOH, add Amberlite IR-120, reflux for 1 h, evaporate to dryness, reconstitute with dichloromethane, chromatograph on a small column of Kieselgel 60 (Merck) with hexane:ethyl acetate 90:10, evaporate to dryness, inject an aliquot of a solution in the mobile phase.

HPLC VARIABLES
Column: 300 × 4 CSP 1 chiral column (Details of column preparation are in paper.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 6.26 (first enantiomer, α = 1.18 (alanine)), k' 4.48 (first enantiomer, α = 1.16 (2-aminobutyric acid)), k' 3.65 (first enantiomer, α = 1.11 (norvaline)), k' 3.21 (first enantiomer, α = 1.14 (valine)), k' 3.26 (first enantiomer, α = 1.08 (norleucine)), k' 3.07 (first enantiomer, α = 1.09 (leucine)), k' 2.84 (first enantiomer, α = 1.09 (isoleucine)), k' 6.27 (first enantiomer, α = 1.02 (phenylglycine)), k' 5.53 (first enantiomer, α = 1.11 (phenylalanine)), k' 7.11 (first enantiomer, α = 1.16 (methionine)), k' 5.61 (first enantiomer, α = 1.00 (proline)), k' 3.27 (first enantiomer, α = 1.02 (N-methylalanine))

KEY WORDS

chiral; derivatization

REFERENCE

Chen,C.-C.; Lin,C.-E. HPLC separation of enantiomers of amino acids and amino alcohols on ionically bonded chiral stationary phases consisting of cyanuric chloride with amino acid and dialkylamine substituents, *J.Chromatogr.Sci.*, **1995**, *33*, 229–235.

SAMPLE

Matrix: solutions

Sample preparation: 400 μL Amino acid solution + 100 μL 1 M pH 8.9 borate buffer + 500 μL 10 mM 2-(9-anthryl)ethyl chloroformate (Eka Nobel) in MeCN, mix, let stand for 5 min, add 1 mL pentane, extract, discard the pentane layer, inject an aliquot of the reaction mixture.

HPLC VARIABLES

Column: 650 × 0.25 5 μm Kromasil C8 (Eka Nobel)

Mobile phase: Gradient. MeCN:100 mM pH 4.1 acetate buffer from 40:60 to 85:15 over 45 min

Flow rate: 0.005-0.01

Injection volume: 0.06-1

Detector: F ex 351 (16 mW Ar laser) em 412 (bandpass filter) or UV 256

CHROMATOGRAM

Retention time: 8.5 (Arg), 11 (Asp, Ser), 12 (Glu), 13 (Thr), 15 (Gly), 17.5 (Ala), 20.5 (Pro), 22 (Met), 24 (Phe), 24.5 (Val), 25.5 (Cys), 26.5 (Cystine), 27.5 (Ile), 28 (Leu), 33.5 (His), 36 (Lys), 39 (Tyr)

Limit of detection: 400 nM (UV), 0.30 nM (F)

KEY WORDS

capillary HPLC; comparison with capillary electrophoresis; derivatization

REFERENCE

Engström,A.; Andersson,P.E.; Josefsson,B.; Pfeffer,W.D. Determination of 2-(9-anthryl)ethyl chloroformate-labeled amino acids by capillary electrophoresis and liquid chromatography with absorbance or fluorescence detection, *Anal.Chem.*, **1995**, *67*, 3018–3022.

SAMPLE

Matrix: solutions

Sample preparation: Mix Vortex 10 μL peptide solution in pyridine:water 50:50 and 10 μL 20 mM reagent in pyridine:water 50:50, heat at 50° for 15 min, wash 3 times with 100 μL porions of n-heptane:dichloromethane 90:10. Remove the aqueous phase and dry it in a centrifugal evaporator at 50° for 15 min, add 30 μL trifluoroacetic acid to the residue, heat at 50° for 10 min, evaporate to dryness under a stream of nitrogen, reconstitute with 20 μL water, extract 3 times with n-heptane:dichloromethane 80:20 (retain the aqueous phase for the next cycle). Combine the extracts and evaporate them to dryness under a stream of nitrogen, reconstitute in MeCN, inject an aliquot. (Perform all reactions under nitrogen. N-Terminal amino acid is derivatized and sequenced. Reagent

was 7-[(N,N- dimethylamino)sulfonyl]-2,1,3-benzoxadiazol-4-yl isothiocyanate and it was prepared as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm ,Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Stir 50 mg DBD-F in 15 mL MeCN and add 100 μL 28% ammonia in water, stir at room temperature overnight, evaporate under reduced pressure, recrystallize from MeCN to give 4-amino-7-N,N-dimethylaminosulfonyl-2,1,3-benzoxadiazole as pale yellow needles (DBD-NH2) (mp 214-7°). Add 1 mL 30% thiophosgene in benzene dropwise to 200 mg DBD-NH2 in 15 mL MeCN, reflux for 5 h, evaporate under reduced pressure, extract the residue twice with 20 mL portions of chloroform. Filter the extracts and evaporate them to dryness, dissolve the residue in chloroform and chromatograph on 15 g silica gel (G-200) with chloroform. Evaporate the eluate to dryness and recrystallize 7-[(N,N-dimethylamino)sulfonyl]-2,1,3-benzoxadiazol-4-yl isothiocyanate from benzene-n-hexane to give yellow-white crystals (mp 122-4°) (Biomed.Chromatogr. 1992, 7, 56).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC J'sphere ODS H-80 (YMC) + 250 × 4.6 5 μm YMC-Pack Ph phenyl (YMC) in series
Mobile phase: MeCN:water 60:40 containing 10 mM formic acid
Flow rate: 0.5
Injection volume: 20
Detector: F ex 387 em 524

CHROMATOGRAM

Retention time: 14 (Ser), 17 (Arg), 17.5 (Thr), 21 (Gly), 23 (Tyr), 24 (Ala), 26 (Asn), 28 (His), 29 (Pro), 30.5 (Asp), 31 (Glu), 32 (Met), 33, 45 (Lys), 36 (Val), 41 (Phe), 46 (Ile), 48 (Leu)
Limit of detection: 200 pmole

KEY WORDS
derivatization

REFERENCE
Matsunaga,H.; Santa,T.; Hagiwara,K.; Homma,H.; Imai,K.; Uzu,S.; Nakashima,K.; Akiyama,S. Development of an efficient amino acid sequencing method using fluorescent Edman reagent 7-[(N,N-dimethylamino)sulfonyl]-2,1,3-benzoxadiazol-4-yl isothiocyanate, *Anal.Chem.*, **1995**, *67*, 4276–4282.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL of an amino acid solution in MeCN:water:triethylamine 50:50:2 with 10 μL 5 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in MeCN, heat at 55° for 10 min, add 480 μL 1 M acetic acid in MeCN:water 50:50, dilute 10-fold with MeCN, inject a 5 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei (TCI America, Portland OR). Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro- 2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°) (Analyst 1995, 120, 385).

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 25:75:0.05 (A) or 30:70:0.05 (B) or 35:65:0.05 (C) or 40:60:0.05 (D) or 45:55:0.05 (E)
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: F ex 490 em 530

CHROMATOGRAM
Retention time: k' 10.71 (D-alanine (A)), k' 11.19 (L-alanine (A)), k' 3.34 (D-cystine (E)), k' 4.03 (L-cystine (E)), k' 10.94 (D-isoleucine (C)), k' 12.30 (L-isoleucine (C)), k' 5.61 (D-

leucine (D)), k' 6.29 (L-leucine (D)), k' 7.91 (D-lysine (D)), k' 9.13 (L-lysine (D)), k' 6.37 (D-methionine (C)), k' 6.92 (L-methionine (C)), k' 12.17 (D-norleucine (C)), k' 13.75 (L-norleucine (C)), k' 4.87 (D-proline (B)), k' 6.06 (L-proline (B)), k' 12.52 (D-phenylalanine (C)), k' 14.90 (L-phenylalanine (C)), k' 7.77 (D-threonine (A)), k' 8.25 (L-threonine (A)), k' 5.95 (D-tryptophan (D)), k' 7.05 (L-tryptophan (D)), k' 9.20 (D-tyrosine (B)), k' 10.00 (L-tyrosine (B)), k' 6.11 (D-valine (C)), k' 6.81 (L-valine (C))

KEY WORDS
derivatization; chiral

REFERENCE
Toyo'oka,T.; Liu,Y.-M. High-performance liquid chromatographic resolution of amino acid enantiomers derivatized with fluorescent chiral Edman reagents, *J.Chromatogr.A*, **1995**, *689*, 23–30.

SAMPLE
Matrix: solutions
Sample preparation: 35 μL Solution of amino acids in water + 35 μL 3.3 mg/mL o-phthalaldehyde in MeOH:water 50:50 + 35 μL 4 mg/mL N-acetyl-L-cysteine in MeOH:water 50:50 + 175 μL 400 mM pH 9.4 potassium borate buffer, mix well, let stand at room temperature for at least 2 min (J.Chromatogr. 1989, 471, 263), neutralize with trifluoroacetic acid, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil 120-C18
Mobile phase: MeOH:buffer 40:60 (Buffer 50 mM ammonium acetate adjusted to pH 6.0 with acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: MS, Finnigan MAT TSQ 70 triple quadrupole, thermospray, first and second quadrupoles in rf-only mode, third quadrupole in scan and mass selective mode, the column effluent was mixed with 1% trifluoroacetic acid in water pumped at 0.25 mL/min, first 5 mL of column effluent was diverted from the detector, source 200°, repeller 120 V, vaporizer 90

KEY WORDS
derivatization; chiral

REFERENCE
van Leuken,R.G.J.; Duchateau,A.L.L.; Kwakkenbos,G.T.C. Thermospray liquid chromatography/mass spectrometry study of diastereomeric isoindole derivatives of amino acids and amino acid amides, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1459–1464.

SAMPLE
Matrix: solutions
Sample preparation: 30 μL 100 mM Amino acid in 1 M HCl + 45 μL 1 M sodium bicarbonate + 500 μL 10 mM reagent in DMSO, heat at 100° for 1 h, add 1.425 mL DMSO, inject a 1-5 μL aliquot. (Prepare reagent as follows. Add 2.14 g L-valinamide hydrochloride in 10 mL water to 2.58 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 35 mL acetone with stirring while maintaining the temperature at 5-10°, add 8 mL 2 M sodium carbonate, stir at 20° for 1 h, evaporate to dryness under reduced pressure, take up the residue in 350 mL ethyl acetate, wash with two 175 mL portions of water, add 10 mL toluene, evaporate to dryness to give N-(4,6-dichloro-[1,3,5]triazin-2-yl)-L-valine amide as a white solid (mp 151-152°). Add 1.64 g L-phenylalaninamide in 10 mL water to 2.64 g N-(4,6-dichloro-[1,3,5]triazin-2- yl)-L-valine amide in 35 mL acetone with stirring at room temperature, add 10 mL 1 M sodium bicarbonate, stir at room temperature for 3 h, evaporate to dryness under reduced pressure, take up the residue in 350 mL ethyl acetate, wash with two 175 mL portions of water, add 10 mL toluene, evaporate to dryness to give

N-[4-((S)-1-carbamoyl-2-methylpropylamino)-6-chloro-[1,3,5]triazin-2-yl]-L-phenylalanine amide (mp 128-130°).)

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil 100 C18
Mobile phase: MeCN:10 mM pH 4 sodium acetate 20:80
Flow rate: 1
Injection volume: 1-5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.90 (L-Glu), 5.89 (D-Glu), 8.26 (L-Pro), 12.76 (D-Pro), 38.28 (L-Phe), 78.14 (D-Phe)

KEY WORDS
derivatization; chiral

REFERENCE
Brückner,H.; Wachsmann,M. Liquid chromatographic separation of amino acid enantiomers on a silica-bonded chiral *s*-triazine column, *J.Chromatogr.A*, **1996**, *728*, 447–454.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 40 μL aliquot of a solution of amino acids in 100 mM HCl with 100 μL 500 mM pH 9.0 potassium borate buffer and 250 μL 0.5 mM 4-phenylazobenzyloxycarbonyl chloride in MeCN, let stand for 5 min, add 100 μL reagent, mix, let stand for 5 min. Remove an 80 μL aliquot and add it to 320 μL MeCN:500 mM pH 4.0 sodium acetate buffer 20:80, mix, inject a 20 μL aliquot. (Prepare reagent by mixing 3 mL n-heptylamine, 15 mL MeCN, and 175 mL 100 mM HCl, pH 7-8. 4-Phenylazobenzyloxycarbonyl chloride can be purchased from Bachem, Bubendorf, Switzerland. Synthesis is as follows. Dissolve 10 g 4-nitrobenzylalcohol in 100 mL MeOH, add 1 mL triethylamine, add 2 g Raney nickel, hydrogenate at room temperature and atmospheric pressure, filter. Evaporate the filtrate to dryness, add benzene (Caution! Benzene is a carcinogen!), evaporate to dryness to remove water, repeat this step to obtain 4-aminobenzyl alcohol as crystals. Mix 7.7 g 4-aminobenzyl alcohol with a solution of 7.4 g nitrosobenzene in 40 mL acetic acid with stirring at 0°, after 3 h filter, wash the solid with dilute acetic acid to obtain 4-phenylazobenzylalcohol (mp 142.5-143°). Dilute the filtrate with a lot of water, filter, extract with hot carbon tetrachloride, crystallize from carbon tetrachloride to obtain more p-phenylazobenzyl alcohol. Dissolve 10.9 g phosgene in 40 mL dioxane, add 5 g p-phenylazobenzylalcohol at 0°, stir at 0° for 15 min, let stand at room temperature for 3 h, filter, evaporate the filtrate to dryness under reduced pressure. Recrystallize the residue from petroleum ether to give 4-phenylazobenzyloxycarbonyl chloride (mp 82-83°) (Helv. Chim. Acta 1958, 41, 491). Alternatively, recrystallize in the cold from n-hexane/ethyl acetate.)

HPLC VARIABLES
Column: 250 × 4 5 μm Grom-Sil 120 ODS-3 CP porous encapsulated polymer-coated spherical particles (Grom, Herrenberg, Germany)
Mobile phase: Gradient. A was MeCN:THF:100 mM pH 6.7 sodium acetate buffer 20:2:78. B was MeCN:THF 98:2. A:B from 100:0 to 95:5 over 15 min, to 85:15 over 18 min, to 75:25 over 24 min, to 50:50 over 10 min, to 0:100 over 1 min, maintain at 0:100 for 7 min, return to initial conditions over 0.1 min, re-equilibrate for 10 min.
Column temperature: 45
Flow rate: 1 for 5 min, to 1.25 over 0.1 min, maintain at 1.25
Injection volume: 20
Detector: UV 320

CHROMATOGRAM
Retention time: 7.75 (Asp), 8.95 (Glu), 18.05 (Ser), 19.56 (Arg), 19.94 (Gly), 20.84 (Thr), 21.45 (Pro), 22.37 (Ala), 26.77 (Val), 29.83 (Tyr), 31.21 (Met), 34.65 (Ile), 35.43 (Leu), 38.11 (Phe), 63.99 (His), 64.42 (Lys)
Limit of detection: 0.5 pmole

KEY WORDS
derivatization

REFERENCE
Kirschbaum,J.; Brückner,H. Amino acid analysis by derivatization with chromogenic 4-phenylazobenzyloxycarbonyl chloride (PAZ-Cl): Comparison of reversed phases, *Chromatographia*, **1996**, *43*, 275–278.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL Amino acid solution in 250 mM pH 8.8 borate buffer + 10 μL 4 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 1.5 min, add 10 μL reagent, mix, let stand for 3.5 min, add 10 mL (?) MeCN:glacial acetic acid 80:20, mix, inject an aliquot. (Reagent was 150 mL 500 mM hydroxylamine hydrochloride containing 340 μL 850 mM NaOH and 10 μL 2-(methylthio)ethanol.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-Hypersil
Mobile phase: Gradient. A was MeOH:water 15:85 containing 30 mM $(NH_4)H_2PO_4$, pH 6.5. B was MeOH:water 15:85. C was MeCN:water 90:10. A:B:C 17:68:15 for 1 min, to 43.2:46:12 over 31 min, to 0:0:100 over 0.05 min, maintain at 0:0:100 for 1.95 min.
Column temperature: 38
Flow rate: 1
Detector: F ex 270 em 316

CHROMATOGRAM
Retention time: 3 (aspartic acid), 3.5 (glutamic acid), 7.5 (hydroxyproline), 9.7 (serine), 10.3 (histidine), 10.6 (glycine), 11.2 (threonine), 11.8 (alanine), 12.5 (proline), 13.5 (tyrosine), 15 (arginine), 16 (valine), 16.5 (methionine), 18.5 (isoleucine), 19 (leucine), 20 (phenylalanine)

KEY WORDS
derivatization

REFERENCE
Ou,K.; Wilkins,M.R.; Yan,J.X.; Gooley,A.A.; Fung,Y.; Sheumack,D.; Williams,K.L. Improved high-performance liquid chromatography of amino acids derivatized with 9-fluorenylmethyl chloroformate, *J.Chromatogr.A*, **1996**, *723*, 219–225.

SAMPLE
Matrix: solutions
Sample preparation: Rapidly mix 1 mL 10 mM dansyl chloride in MeCN with 2 mL amino acid solution in buffer, sonicate for 10 min in the dark, let stand at room temperature in the dark for 40 min, inject a 25 μL aliquot. (Buffer was 40 mM lithium carbonate adjusted to pH 9.5 with HCl.)

HPLC VARIABLES
Guard column: 70 × 2 Partisil ODS
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 17:83 containing 0.5 mM tris(2,2'-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3^{2+}$)

Flow rate: 1
Injection volume: 25
Detector: chemiluminescence following post-column electrochemical oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$. The column effluent flowed through a Bioanalytical Systems BAS 100A electrochemical detector with a Pt electrode at 1250 mV (relative to an Ag/AgCl reference electrode) to the chemiluminescence detector.

CHROMATOGRAM

Retention time: 13 (Glu), 23 (Asn), 32 (Ser), 40 (Thr), 46 (Gly), 52 (Ala)
Limit of detection: 100 nM

KEY WORDS

derivatization

REFERENCE

Skotty,D.R.; Lee,W.-Y.; Nieman,T.A. Determination of dansyl amino acids and oxalate by HPLC with electrogenerated chemiluminescence detection using tris(2,2'-bipyridyl)ruthenium(II) in the mobile phase, *Anal.Chem.*, **1996**, *68*, 1530–1535.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate 25 μL of an amino acid solution to dryness under a stream of nitrogen at 50°, add MeCN, evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL MeCN:MeOH:triethylamine 10:5:2, add 3 μL butylisothiocyanate, sonicate for 1 min, heat at 40° for 30 min, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeCN, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 20 mM ammonium acetate, filter (0.25 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 50 mM ammonium acetate adjusted to pH 6.7 with phosphoric acid. B was MeCN:MeOH:THF:20 mM Na_2HPO_4 50:2.5:0.75:46.75. C was MeCN:water 70:30. A:B:C from 100:0:0 to 85:15:0 over 8 min, to 70:20:10 over 6 min, to 60:20:20 over 6 min, to 30:20:50 over 5 min, to 10:20:70 over 5 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 20 min.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 8 (Asp), 9 (Glu), 12 (Hyp), 14 (Asn, Ser), 14.3 (Gly), 14.6 (Gln), 15.6 (His), 15.9 (Thr), 16.2 (Ala), 16.6 (Arg), 17 (Pro), 20.3 (Tyr), 20.6 (Val), 22 (Met), 23 (Ile), 23.5 (Leu), 24 (cystine), 25.3 (Phe), 26 (Trp), 26.5 (Lys), 27.7 (cysteine)
Internal standard: norleucine (24.5)
Limit of detection: 3.9 pmole

KEY WORDS

derivatization; comparison with phenylisothiocyanate derivatization

REFERENCE

Woo,K.L.; Hwang,Q.C.; Kim,H.S. Determination of amino acids in the foods by reversed-phase high-performance liquid chromatography with a new precolumn derivative, butylthiocarbamyl amino acid, compared to the conventional phenylthiocarbamyl derivatives and ion-exchange chromatography, *J.Chromatogr.A*, **1996**, *740*, 31–40.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 50 mM aqueous solution with 20 μL 1 M sodium bicarbonate, add 100 μL 1% Marfey's reagent in acetone, vortex, heat at 37° for 1 h, add 20 μL 1 M HCl, add 810 μL MeCN, inject a 1 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-AR (Nacalai Tesque)

Mobile phase: Gradient. MeCN:buffer from 15:85 to 45:55 over 5 min, re-equilibrate at initial conditions for 15 min. (Buffer was 100 mM ammonium acetate in water, adjusted to pH 3 with trifluoroacetic acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 1

Detector: UV 340

CHROMATOGRAM

Retention time: 3.7 (D-β-threo-hydroxyaspartic acid), 3.8 (L-β-threo-hydroxyaspartic acid), 6.0 (D-histidine (mono-derivative)), 6.1 (D-β-erythro-hydroxyaspartic acid), 6.7 (L-asparagine), 6.7 (L-histidine (mono-derivative)), 6.9 (L-β-erythro-hydroxyaspartic acid), 7.3 (D-asparagine), 8.2 (L-aspartic acid), 8.5 (D-ornithine (mono-derivative)), 8.8 (L-glutamine), 9.0 (L-homoserine), 9.2 (L-serine), 9.3 (L-arginine), 9.4 (L-ornithine (mono-derivative)), 9.6 (D-glutamine), 9.6 (D-arginine), 9.8 (D-aspartic acid), 9.9 (D-serine), 10.0 (D-citrulline), 10.4 (L-allo-threonine), 10.4 (L-threonine), 10.5 (D-homoserine), 10.6 (L-lysine (mono-derivative)), 10.7 (L-citrulline), 10.8 (D-lysine (mono-derivative)), 10.8 (L-glutamic acid), 12.3 (D-allo-threonine), 12.3 (L-O-methylserine), 13.1 (D-glutamic acid), 14.8 (D-threonine), 14.8 (L-alanine), 15.7 (L-proline), 17.2 (D-O-methylserine), 17.9 (L-2-amino-n-butyric acid), 18.2 (D-proline), 19.1 (D-alanine), 20.2 (L-methionine), 21.9 (L-histidine (bis-derivative)), 23.2 (L-valine), 23.8 (D-2-amino-n-butyric acid), 24.0 (L-norvaline), 25.3 (D-histidine (bis-derivative)), 26.4 (D-methionine), 27.7 (L-phenylalanine), 28.7 (L-isoleucine), 29.6 (L-leucine), 29.7 (D-valine), 29.9 (L-norleucine), 30.4(D-norvaline), 31.4 (L-ornithine (bis-derivative)), 31.4 (L-lysine (bis-derivative)), 33.2 (D-phenylalanine), 34.5 (D-lysine (bis-derivative)), 34.5 (D-ornithine (bis-derivative)), 35.3 (D-isoleucine), 35.9 (D-leucine), 36.5 (D-norleucine), 37.8 (L-tyrosine (bis-derivative)), 42.6 (D-tyrosine (bis-derivative))

KEY WORDS

derivatization; chiral

REFERENCE

Fujii,K.; Ikai,Y.; Mayumi,T.; Oka,H.; Suzuki,M.; Harada,K.-I. A nonempirical method using LC/MS for determination of the absolute configuration of constituent amino acids in a peptide: Elucidation of limitations of Marfey's method and of its separation mechanism, *Anal.Chem.*, **1997**, *69*, 3346–3352.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL aliquot of a 10-25 μg/mL solution of amino acids in water with 100 μL buffer, add 50 μL 5% 2,4-dinitrofluorobenzene in MeCN, vortex, heat at 50° for 1 h, add 250 μL 1% acetic acid, add 500 μL MeCN:water 50:50, inject a 20 μL aliquot. (Buffer was 100 mM sodium bicarbonate adjusted to pH 9.5 with 100 mM sodium carbonate.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm 100 Å Kromasil (EKA Nobel) derivatized with a chiral quinine carbamate (J. Chromatogr.A 1996, 741, 33)

Mobile phase: MeCN:THF:110 mM ammonium acetate 44.55:0.45:55, adjusted to pH 5.5 with glacial acetic acid

Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 390

CHROMATOGRAM

Retention time: 13.11 (L-Thr), 15.64 (L-Pro), 18.29 (D-Thr), 18.90 (L-Ala), 19.93 (L-Val), 21.47 (D-Pro), 22.43 (D-Ala), 23.79 (L-Leu), 26.04 (D-Val), 30.37 (D-Leu), 33.47 (L-Phe), 40.93 (D-Phe), 48.00 (L-Trp), 66.03 (D-Trp)

KEY WORDS

derivatization; chiral

REFERENCE

Lammerhofer,M.; Di Eugenio,P.; Molnar,I.; Lindner,W. Computerized optimization of the high-performance liquid chromatographic enantioseparation of a mixture of 4-dinitrophenyl amino acids on a quinine carbamate-type chiral stationary phase using DRYLAB, *J.Chromatogr.B*, **1997**, *689*, 123–135.

SAMPLE

Matrix: soybean meal
Sample preparation: Mix 200 mg soybean meal with 15 mL 0.1% phenol in 6 M HCl, pump out the tube then flush with nitrogen 3 times, heat at 145° for 4 h, filter, evaporate to dryness under reduced pressure at 50°, reconstitute with 50 mL 10 mM HCl. Add a 5 mL aliquot to a 100 × 13 Dowex 5X8 cation exchange column, elute with 4 M ammonia. Evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 50 mL 10 mM HCl. Evaporate a 500 μL aliquot to dryness under a stream of nitrogen at 50°, add 30 μL MeCN, evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL 2.5 mM L-norleucine in MeCN:MeOH:triethylamine 10:5:2, add 3 μL benzylisothiocyanate, heat at 50° for 30 min, evaporate to dryness under a stream of nitrogen with simultaneous vacuum pump evacuation at room temperature, reconstitute the residue in 50 μL MeCN, evaporate to dryness as before, reconstitute the residue in 1 mL initial mobile phase, filter (0.2 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeOH:THF:20 mM NaH_2PO_4 5:1.5:93.5, adjusted to pH 6.8 with phosphoric acid. B was MeCN:MeOH:THF:20 mM NaH_2PO_4 50:2.5:0.75:46.75, adjusted to pH 6.8 with phosphoric acid. C was MeCN:water 70:30. A:B:C from 100:0:0 to 80:20:0 over 10 min, to 76:20:4 over 5 min, to 70:20:10 over 5 min, to 50:30:20 over 10 min, to 30:35:35 over 10 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 20 min.
Column temperature: 40
Flow rate: 1.2
Injection volume: 10
Detector: UV 246

CHROMATOGRAM

Retention time: 4 (Asp), 4.5 (Glu), 7 (Hyp), 9.5 (Asn), 10 (Gln), 10.5 (Ser), 11 (Gly), 11.5 (His), 12 (Pro), 12.5 (Arg), 13 (Thr), 13.5 (Ala), 17.5 (ammonia), 19 (Asp), 20 (Val), 20.5 (Tyr), 22 (Met), 25 (Ile), 25.5 (Leu), 27.5 (cystine), 28 (Phe), 29.5 (Trp), 32 (Lys), 34.5 (cysteine)
Internal standard: L-norleucine (26)
Limit of detection: 3.9 pmole

KEY WORDS

derivatization; SPE; comparison with phenylisothiocyanate derivatization

REFERENCE

Woo,K.-L.; Ahan,Y.-K. Determination of protein amino acids as benzylthiocarbamyl derivatives compared with phenylthiocarbamyl derivatives by reversed-phase high-performance liquid chromatography, ultraviolet detection and precolumn derivatization, *J.Chromatogr.A*, **1996**, *740*, 41–50.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Polytron for >10 mg; Kontes micro-ultrasonic cell disrupter for <10 mg) tissue with 40 volumes 25 μg/mL β-aminoisobutyric acid in EtOH:water:glacial acetic acid 75:20:5, centrifuge at 4° at 25000 g for 20 min. Remove a 50 μL aliquot of the supernatant and evaporate it to dryness under reduced pressure, suspend the residue in 100 μL 100 mM sodium bicarbonate by sonicating or vortexing, add 200 μL 1.25 mg/mL dansyl chloride in acetone, vortex, heat at 90° for 30 min, centrifuge at 5000 g for 20 min, inject a 4 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: MeCN:water:phosphoric acid 13:87:0.15

Flow rate: 1

Injection volume: 4

Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.61 (cysteic acid), k' 0.93 (glutathione), k' 0.98 (ethanolamine), k' 1.24 (asparagine), k' 1.26 (taurine), k' 1.30 (methionine), k' 1.71 (ammonia), k' 1.58 (glutamine), k' 1.94 (cystathionine), k' 1.95 (leucine), k' 1.95 (lysine), k' 1.96 (isoleucine), k' 2.01 (cysteine), k' 2.14 (proline), k' 2.43 (homocarnosine), k' 2.45 (urea), k' 2.54 (arginine), k' 2.54 (hydroxyproline), k' 2.70 (glutamic acid), k' 2.86 (aspartic acid), k' 2.86 (serine), k' 3.65 (threonine), k' 4.01 (glycine), k' 4.55 (norvaline), k' 5.91 (alanine), k' 5.93 (valine), k' 6.48 (GABA), k' 8.58 (6-aminocaproic acid), k' 11.42 (α-aminobutyric acid), k' 16.22 (tryptophan), k' 22.13 (tyrosine)

Internal standard: β-aminoisobutyric acid (k' 9.25)

Limit of quantitation: 10 pmole

KEY WORDS

rat; brain; derivatization

REFERENCE

Saller,C.F.; Czupryna,M.J. γ-Aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives, *J.Chromatogr.*, **1989**, *487*, 167–172.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize mouse liver with chloroform:MeOH 2:1, extract the homogenate with water. Lyophilize the aqueous phase, reconstitute with water. Remove a 100 μL aliquot, add 10 μL 10 mM NaOH, add 100 μL 100 mM pH 9.0 borate buffer, add 100 μL 1 mM dansyl chloride in MeCN, vortex, heat at 40° for 45 min, cool to room temperature, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 LiChrosorb RP-18

Mobile phase: Gradient. A was MeCN:10 mM pH 7.0 phosphate buffer 10:90. B was MeCN:10 mM pH 7.0 phosphate buffer 50:50. A:B from 75:25 to 30:70 over 20 min, maintain at 30:70 for 10 min, return to initial conditions over 0.1 min, re-equilibrate for 10 min.

Flow rate: 3

Injection volume: 75

Detector: F ex 330 em 565, UV 254

CHROMATOGRAM
Retention time: 8.24 (tyrosine), 11.9 (tryptophan)

KEY WORDS
derivatization; mouse; liver

REFERENCE
Manwaring,J.D.; Csallany,A.S. Identification of vitamin E-dependent water soluble fluorescent compounds in mouse tissues, *Lipids*, **1990**, *25*, 22–26.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (glass/PTFE homogenizer) tissue in 320 mM sucrose, mix 60 μL homogenate with 100 μL 400 mM perchloric acid, centrifuge at 10000 g for 10 min. Remove a 100 μL aliquot of the supernatant and add it to 65 μL 2 M potassium bicarbonate, centrifuge at 10000 g for 10 min. Remove a 10 μL aliquot of the supernatant and mix it with 10 μL 1 mM α-aminoadipic acid, 20 μL 25 mM sodium bicarbonate, and 80 μL 4 mM dabsyl chloride in MeCN, vortex thoroughly, heat at 70° for 12 min, add 380 μL 20% acetic acid, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher C18
Mobile phase: Gradient. A was DMF:25 mM pH 6.4 sodium acetate buffer 4:96. B was MeCN. A:B 85:15 for 2 min, to 70:30 over 28 min, to 59:41 over 5 min, to 46:54 over 2 min, to 43:57 over 2 min, to 33:67 over 2 min, to 10:90 over 1 min, return to initial conditions over 2 min, re-equilibrate for 11 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 436

CHROMATOGRAM
Retention time: 10 (aspartate), 11 (glutamate), 19 (glutamine), 20 (serine), 21 (threonine), 22 (glycine), 23 (alanine), 25 (arginine, taurine), 26.5 (proline, gamma-aminobutyric acid), 27.5 (valine), 30 (methionine), 31.5 (isoleucine) 32.5 (leucine), 35 (phenylalanine), 37.5 (cystine), 38.5 (ammonia), 40.5 (lysine), 41 (histidine), 42 (tyrosine)
Internal standard: α-aminoadipic acid (12)

KEY WORDS
derivatization; brain

REFERENCE
Watanabe,A.; Semba,J.; Kurumaji,A.; Kumashiro,S.; Toru,M. Measurement of glutamate, aspartate and glycine and its potential precursors in human brain using high-performance liquid chromatography by pre-column derivatization with dimethylaminoazobenzene sulphonyl chloride, *J.Chromatogr.*, **1992**, *583*, 241–245.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize oocytes with 3 volumes of water, add 9 volumes ice-cold MeOH, centrifuge at 4° at 2000 g for 10 min. Evaporate the supernatant to dryness under reduced pressure, resuspend in 100 μL MeOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 1-10 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10 per oocyte, let stand at room temperature for 15 min, dry under vacuum, reconstitute with 50 mM pH 6.8 ammonium acetate buffer, inject an aliquot.

HPLC VARIABLES
Column: 100 × 5 Nova-Pak C18
Mobile phase: Gradient. A was 50 mM pH 6.8 ammonium acetate buffer. B was MeCN: 100 mM ammonium acetate 50:50. A:B 100:0 for 5 min, to 90:10 over 1 min, maintain at 90:10 for 4 min, to 50:50 over 15 min, maintain at 50:50 for 5 min.
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6 (Asp), 3.5 (Glu), 9 (Ser), 10 (Gly), 12.5 (Asn), 13.3 (Gln), 15 (Thr), 15.4 (Ala, His), 17 (Pro), 20.6 (Tyr), 20.7 (Val), 21.7 (Met), 23 (Ile), 23.3 (Leu), 25 (Phe), 26 (Trp), 27.5 (Lys)

KEY WORDS
derivatization; oocytes

REFERENCE
O'Connor,C.M. Analysis of aspartic acid and asparagine metabolism in *Xenopus laevis* oocytes using a simple and sensitive HPLC method, *Mol.Reprod.Dev.*, **1994**, *39*, 392–396.

SAMPLE
Matrix: tissue culture
Sample preparation: Dilute tissue culture 50-fold with initial mobile phase, filter (0.45 μm), remove a 10 μL aliquot of the filtrate and add it to 20 μL reagent, mix, inject. (Prepare reagent by mixing 12 mL Fluoraldehyde (Pierce) with 10 μL mercaptoethanol. Fluoraldehyde contains o-phthalaldehyde, mercaptoethanol, and Brij-35.)

HPLC VARIABLES
Guard column: C18
Column: Resolve C18 (Waters)
Mobile phase: Gradient. A was MeOH:THF:50 mM pH 7.5 sodium acetate buffer containing 50 mM sodium phosphate 2:2:96. B was MeOH:water 65:35. A:B from 100:0 to 0:100 over 47 min, maintain at 0:100 for 15 min, re-equilibrate at initial conditions for 30 min.
Flow rate: 1.5
Detector: F ex 334 (filter) em 425 (filter)

CHROMATOGRAM
Retention time: 4.6 (Asp), 8.3 (Glu), 17.6 (Ser), 20.3 (Gln), 20.5 (His), 23.3 (Gly), 24.6 (Thr), 26.0 (Arg), 29.5 (Ala), 32.9 (Tyr), 40.0 (Met), 40.5 (Val), 42.5 (Phe), 44.7 (Ile), 45.8 (Leu), 52.0 (Lys)

KEY WORDS
derivatization; paper contains discussion of ways to increase column life

REFERENCE
Krok,K.A.; Seaver,S.S. Realities of automating OPA HPLC amino acid analyses, *BioTechniques*, **1991**, *10*, 664–670.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize eyes or nervous tissue with a 9-fold excess of 8% perchloric acid, centrifuge at 0° at 20000 g for 10 min. Neutralize the supernatant with solid potassium bicarbonate, centrifuge. Mix a 100 μL aliquot of the supernatant with 400 μL 300 mM pH 11.0 borate/NaOH buffer and 500 μL 18 mM (+)-1-(9-fluorenyl)ethyl chloroformate in acetone, let stand at room temperature for 45 s, wash twice with 2 mL portions of pentane, filter (0.45 μm) the aqueous phase, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 4.6 Nucleosil C18

Column: 250 × 4.6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: Gradient. A was MeCN:THF:15 mM citric acid containing 10 mM tetramethylammonium chloride 10:75:15, adjusted to pH 2.0. B was MeCN:THF:15 mM citric acid containing 10 mM tetramethylammonium chloride 20:10:70, adjusted to pH 5.3. C was MeCN:THF:15 mM citric acid containing 10 mM tetramethylammonium chloride 60:10:30, adjusted to pH 6.2. A:B:C from 100:0:0 to 15:85:0 over 3 min, to 13:87:0 over 12 min, to 0:30:70 over 70 min, to 0:0:100 over 0.1 min, maintain at 0:0:100 for 9.9 min. (Adjust pH with 6 M HCl or 6 M NaOH.)
Column temperature: 55
Flow rate: 0.7
Injection volume: 20
Detector: F ex 260 em 310

CHROMATOGRAM

Retention time: 18.5 (D-Arg), 19.5 (L-Arg), 22.5 (taurine), 25.4 (D-Asn), 26 (L-Asn), 26.5 (D-Gln), 27.5 (L-Gln), 29 (D-citrulline), 29.3 (L-citrulline), 31.5 (D-Hyp, L-Ser, D-Ser), 32 (L-Hyp), 32.5 (D-Asp), 33.5 (L-Asp), 36 (D-Glu), 36.5 (L-Glu), 37 (D-Thr), 37.5 (L-Thr), 38.5 (Gly), 41.5 (L-α-aminoadipic acid), 45 (L-Pro), 48 (D-Ala, L-Ala), 50.5 (L-Pro, D-Pro), 52 (gamma-aminobutyric acid), 55 (D-β-aminobutyric acid, L-β-aminobutyric acid, L-α-aminobutyric acid), 57 (D-Met), 58 (L-Met), 62.5 (D-Val), 63.5 (L-Val), 65.5 (D-Phe), 66.5 (L-Phe), 71 (D-Ile), 71.5 (D-Leu), 72 (L-Ile), 72.5 (L-Leu), 75 (D-cystine), 76.5 (cystathionine), 77 (L-cystine), 80.5 (D-Hyl, L-Hyl), 88.5 (D-ornithine), 90 (L-ornithine), 91.5 (D-Lys), 92.5 (L-Lys)
Limit of detection: <1 pmole

KEY WORDS

derivatization; chiral; compounds which co-elute under these conditions can be resolved by altering mobile phase conditions.; crab; lobster; prawn; crayfish; nervous tissue; eye

REFERENCE

Okuma,E.; Abe,H. Simultaneous determination of D- and L-amino acids in the nervous tissues of crustaceans using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and reversed-phase ion-pair high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *660*, 243–250.

SAMPLE

Matrix: urine
Sample preparation: Freeze urine at -20°, thaw, stir thoroughly, allow to settle. 600 μL Supernatant + 600 μL concentrated HCl, heat at 110 ± 5° for 18 h, cool, filter, dilute (if necessary). Evaporate a 25-200 μL aliquot to dryness under reduced pressure, reconstitute with 30 μL water:EtOH:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 50 μL phenyl isothiocyanate:EtOH:triethylamine:water 10:70:10:10, vortex, let stand for 10 min, evaporate to dryness under reduced pressure, reconstitute with 500 μL buffer, filter, inject an aliquot. (Buffer was MeCN:10 mM NaH_2PO_4 containing 2 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) 1.8:99.2, pH 6.0.)

HPLC VARIABLES

Guard column: Guard-Pak (Waters)
Column: 250 × 4 5 μm octadecyl (IBM)
Mobile phase: Gradient. A was MeCN:10 mM NaH_2PO_4 containing 2 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) 1.8:99.2, pH 6.0. B was MeCN:water 60:40. A:B 100:0 for 7 min, to 0:100 over 1 min, maintain at 0:100 for 2 min, return to initial conditions over 1 min, re-equilibrate for 7 min
Column temperature: 34 or 43
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM

Retention time: 2.60 (O-phosphoserine), 2.77 (aspartic acid), 3.12 (glutamic acid), 4.13 (α-aminoadipic acid), 5.10 (hydroxyproline), 6.22 (phosphoethanolamine), 6.74 (asparagine), 6.90 (serine), 7.67 (glycine), 7.75 (glutamine), 8.5 (homoserine), 8.72 (sararcosine), 9.24 (β-alanine), 9.25 (anserine), 9.54 (glycerophosphoryl ethanolamine), 10.57 (taurine), 11.57 (citrulline), 12.50 (threonine), 12.84 (gamma-aminobutyric acid), 13.53 (alanine), 14.29 (β-aminoisobutyric acid), 15.06 (histidine), 16.31 (proline), 17.47 (carnosine)

Limit of quantitation: 250 pmoles

KEY WORDS

derivatization

REFERENCE

Lippincott,S.; Chesney,R.W.; Friedman,A.; Pityer,R.; Barden,H.; Mazess,R.B. Rapid determination of total hydroxyproline (HYP) in human urine by HPLC analysis of the phenylisothiocyonate (PITC)-derivative, *Bone*, **1989**, *10*, 265–268.

SAMPLE

Matrix: urine

Sample preparation: 80 μL Urine + 20 μL 10 mM N-methylalanine containing 1 mM norvaline, mix, filter (Ultrafree-MC) while centrifuging at 5000 g for 30 min. Remove a 6 μL aliquot of the ultrafiltrate and add it to 5 μL 0.5% 3-mercaptopropionic acid in 1 M pH 10.4 borate buffer, mix, add 1.5 μL 120 mM iodoacetic acid in 140 mM NaOH, mix, add 5 μL reagent, mix, add 2 μL 9-fluorenylmethyl chloroformate, mix, add 2.5 μL 1 M acetic acid, mix, inject the whole amount. (Reagent was 20 mg/mL o-phthalaldehyde in MeOH:500 mM pH 10.4 borate buffer:3-mercaptopropionic acid 10:88:2.)

HPLC VARIABLES

Guard column: 20 × 4 5 μm ODS Hypersil

Column: 300 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 60 mM pH 6.86 sodium acetate buffer containing 0.044% triethylamine. B was MeCN:MeOH:100 mM pH 5.45 sodium acetate buffer 74.5:4.5:21. A:B from 100:0 to 94.4:5.6 over 1 min, to 93.8:6.2 over 6 min, maintain at 93.8:6.2 for 2 min, to 92.3:7.7 over 12 min, maintain at 92.3:7.7 for 7 min, to 92:8 over 7 min, to 90.8:9.2 over 4 min, to 90.5:9.5 over 3 min, to 84:16 over 6 min, maintain at 84:16 for 1 min, to 82:18 over 1 min, to 78:22 over 20 min, to 72:28 over 7 min, to 68:32 over 8 min, to 0:100 over 9 min, maintain at 0:100 for 5 min, return to initial conditions over 1 min.

Column temperature: 40

Flow rate: 0.8

Injection volume: 22

Detector: F ex 340 em 450 for 79.5 min, F ex 260 em 315 for 1.5 min, F ex 340 em 450 for 6 min, F ex 260 em 315 for 13 min

CHROMATOGRAM

Retention time: 5.5 (Asp), 6.5 (Glu), 8 (Cys (S-carboxymethylated)), 9 (Aad), 10 (Asn), 11 (Ser), 11.5 (Homocysteine (S-carboxymethylated)), 15 (Gln), 17.5 (His), 18 (Gly), 20 (Thr), 25 (Cit), 26 (1-Methylhistidine), 28 (β-Alanine), 29.5 (Arg), 30.5 (3-Methylhistidine), 33 (Ala), 35 (Tau), 38.5 (Ans), 39 (Car), 40 (β-aminoisobutyric acid), 41 (gamma-aminobutyric acid), 52 (Tyr), 52.5 (Abu), 57 (Etn), 60 (Val), 61.5 (Met), 65 (Cysta), 70 (Trp), 71.5 (Ile), 72.5 (Phe), 75 (Hyl), 76.5 (Leu), 80 (Hyp), 83.5 (Lys), 85 (Orn), 90 (Sar), 91 (Pro)

Internal standard: norvaline (62.5), N-methylalanine (91.5)

Limit of detection: 50-500 fmole

Limit of quantitation: 10 μM

KEY WORDS

derivatization; ultrafiltrate

REFERENCE
Carducci,C.; Birarelli,M.; Leuzzi,V.; Santagata,G.; Serafini,P.; Antonozzi,I. Automated method for the measurement of amino acids in urine by high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *729*, 173–180.

SAMPLE
Matrix: vegetables
Sample preparation: Extract 100 g potato tubers with 100 mL boiling water for 2 h, centrifuge at 5000 g for 10 min, filter (0.45 μm) the supernatant, dilute 100 μL of the filtrate with 900 μL 111 ng/mL norvaline in water, add a 1 μL aliquot to 5 μL 0.4 N pH 10.4 potassium borate buffer and 1 μL reagent, mix, add 1 μL 2.5 mg/mL 9-fluorenylmethyl chloroformate in anhydrous MeCN, mix, inject the whole amount. (Prepare reagent by dissolving 10 mg o-phthalaldehyde in 100 μL MeOH, make up to 1 mL with 0.4 N pH 10.4 borate buffer, add 20 μL 3-mercaptopropionic acid. Derivatization was performed automatically and took 5 min. o-Phthalaldehyde derivatized primary amino acids and 9-fluorenylmethyl chloroformate derivatized secondary amino acids (proline and hydroxyproline).)

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 4 3 μm Hypersil ODS
Mobile phase: Gradient. A was THF:18 mM sodium acetate containing 0.02% triethylamine (adjusted to pH 7.2 with 1% acetic acid) 0.3:99.7. B was MeCN:MeOH:100 mM pH 7.2 sodium acetate 40:40:20. A:B from 100:0 to 94:6 over 0.5 min, to 80:20 over 2.5 min, to 50:50 over 3.5 min, to 25:75 over 1.5 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Column temperature: 40
Flow rate: 1.4
Injection volume: 8
Detector: F ex 340 em 450, after 6.8 min F ex 264 em 313

CHROMATOGRAM
Retention time: 0.709 (aspartic acid), 0.831 (glutamic acid), 1.747 (asparagine), 1.871 (serine), 2.160 (glutamine), 2.286 (histidine), 2.439 (glycine), 2.602 (threonine), 3.305 (alanine), 3.446 (arginine), 4.185 (tyrosine), 5.113 (valine), 5.221 (methionine), 5.657 (tryptophan), 5.835 (phenylalanine), 5.928 (isoleucine), 6.224 (leucine), 6.435 (lysine), 6.903 (hydroxyproline), 7.982 (proline)
Internal standard: norvaline (5.394)
Limit of quantitation: 20 pmole

KEY WORDS
derivatization; potato tubers

REFERENCE
Bartók,T.; Szalai,G.; Lorincz,Z.; Börcsök,G.; Sági,F. High-speed RP-HPLC/FL analysis of amino acids after automated two-step derivatization with o-phthaldialdehyde/3-mercaptopropionic acid and 9-fluorenylmethyl chloroformate, *J.Liq.Chromatogr.*, **1994**, *17*, 4391–4403.

SAMPLE
Matrix: yogurt
Sample preparation: 15 g Yogurt + 45 mL MeOH:water 80:20, stir for 10 min, centrifuge at 1630 g. Remove the supernatant and evaporate it to about 10 mL under reduced pressure, add 10 mL of a saturated solution of picric acid, mix, centrifuge at 1630 g. Remove the supernatant and wash it twice with 20 mL portions of light petroleum (bp 40-60°): diethyl ether 50:50, add the aqueous phase to a 50 × 10 column of Dowex 50W-X8 cation-exchange resin, wash with water, elute with 30 mL 2 M aqueous ammonia, evaporate the eluate to dryness, reconstitute with 500 μL 133 mM pH 10.4 borate buffer. Remove a 4

μL aliquot and add it to 2 μL 5 mg/mL o-phthaldialdehyde in 133 mM pH 10.4 borate buffer, add 2 μL 8 mg/mL N-acetyl-L-cysteine in 133 mM pH 10.4 borate buffer, mix for 3 min, inject an aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. A was 40 mM pH 6.5 sodium acetate. B was MeCN. A:B from 100: 0 to 82:18 over 1 h.
Column temperature: 26
Flow rate: 0.9
Detector: UV 338

CHROMATOGRAM

Retention time: 3.8 (D-Asp), 4.1 (L-Asp), 7.4 (L-Glu), 8.0 (D-Glu), 12.3 (L-Ser), 12.7 (L-Asn), 12.9 (D-Ser), 13.7 (D-Asn), 16.5 (L-Gln), 18.0 (D-Gln), 18.2 (D,L-Thr), 18.4 (Gly), 21.0 (L-His), 21.6 (D-His), 25.4 (L-Ala), 26.0 (D-Ala), 27.2 (L-Arg), 28.8 (D-Arg), 36.5 (L-Tyr), 38.9 (D-Tyr), 39.5 (L-Val), 43.6 (D-Val), 44.1 (L-Met), 45.1 (D-Met), 48.8 (L-Ile), 51.5 (L-Trp), 52.6 (D-Ile), 53.2 (D-Trp), 53.6 (D-Phe), 54.1 (L-Phe), 55.1 (L-Leu), 55.7 (D-Leu), 59.3 (L-Lys), 59.7 (D-Lys)

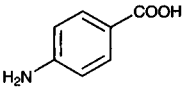
KEY WORDS

derivatization; chiral; SPE; comparison with derivatization procedures using other thiols

REFERENCE

Brückner,H.; Wittner,R.; Godel,H. Automated enantioseparation of amino acids by derivatization with *o*-phthaldialdehyde and N-acylated cysteines, *J.Chromatogr.*, **1989**, *476*, 73–82.

Aminobenzoic acid



Molecular formula: $C_7H_7NO_2$
Molecular weight: 137.14
CAS Registry No.: 150-13-0
Merck Index: 443

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma or urine + 200 μL MeCN, vortex for a few s, centrifuge at 800 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:0.04% pH 2.5 ± 0.05 phosphoric acid 3.5:96.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 8
Internal standard: p-aminobenzoic acid

OTHER SUBSTANCES

Extracted: iothalamate, p-aminohippuric acid

KEY WORDS

plasma; dog; human; p-aminobenzoic acid is IS

REFERENCE

Prueksaritanont,T.; Chen,M.L.; Chiou,W.L. Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids, *J.Chromatogr.*, **1984**, *306*, 89–97.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 50 μL MeCN, vortex for 15 s, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 8 10 μm μBondapak C18
Mobile phase: 67 mM pH 2.8 phosphate buffer containing 5 mM tetrabutylammonium phosphate (low-UV Pic A Reagent, Waters) (Buffer was KH_2PO_4:Na_2HPO_4 97.5:2.5.)
Flow rate: 2
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 13.0
Internal standard: p-aminobenzoic acid

OTHER SUBSTANCES

Extracted: p-aminohippuric acid

KEY WORDS
serum; p-aminobenzoic acid is IS

REFERENCE
Jenny,P.D.; Weber,A.; Smith,A.L. Quantitation of p-aminohippuric acid in biological fluids by high-performance liquid chromatography and dual-wavelength ultraviolet detection, *J.Chromatogr.*, **1989**, *490*, 213–218.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 150 μL salicylic acid in 1 M perchloric acid, centrifuge at 10000 g for 5 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Cosmosil MS-C18
Mobile phase: MeCN:water:glacial acetic acid 3:100:1, adjusted to pH 4.0 with 10 M NaOH
Column temperature: 40
Flow rate: 1.5
Injection volume: 30
Detector: F ex 270 em 350

CHROMATOGRAM
Retention time: 8.32
Internal standard: salicylic acid (17)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, p-acetamidobenzoic acid, p-acetamidohippuric acid, p-aminohippuric acid

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Song,D.J.; Hsu,K.Y. Determination of p-aminobenzoic acid and its metabolites in rabbit plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1996**, *677*, 69–75.

SAMPLE
Matrix: cell cultures
Sample preparation: Condition a cyclohexyl-bonded silica Bond-elut SPE cartridge with 2 mL MeOH and 2 mL water. Centrifuge cell cultures at 6000 g at 4° for 15 min, add 100 μL supernatant and 100 μL 2 μg/mL sulfamerazine to the SPE cartridge, wash with 1 mL water, elute with 1.5 mL MeOH. Evaporate the eluate to dryness under a stream of air at 60°, reconstitute the residue in 100 μL water, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS Hypersil
Mobile phase: MeOH:10 mM pH 2.5 phosphate buffer 5:95 containing 40 mM tetrabutylammonium bromide
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

Internal standard: sulfamerazine (7.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, trimethoprim, dibromopropamidine isethionate

KEY WORDS
SPE

REFERENCE
Taylor,R.B.; Richards,R.M.E.; Xing,D.K.-I. Determination of antibacterial agents in microbiological cultures by high-performance liquid chromatography, *Analyst*, **1990**, *115*, 797–799.

SAMPLE
Matrix: perfusate
Sample preparation: Adjust pH of 5-10 mL perfusate to 5 with 180 μL 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water containing 30 μL/L triethylamine, adjusted to pH 2.3 with phosphoric acid 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Extracted: procaine, aminohippuric acid, 4-acetamidobenzoic acid

KEY WORDS
rabbit; chinchilla; pharmacokinetics

REFERENCE
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, *22*, 1357–1366.

SAMPLE
Matrix: perfusate
Sample preparation: Prepare ultrafiltrate from 200 μL perfusate using an Ultrafree-MC unit with a 30000 MW cut-off (Millipore) with centrifuging at 2000 g for 20 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: Ultrasphere C18
Mobile phase: MeCN:water:acetic acid:triethylamine 12:88:1:0.05
Flow rate: 1.2
Injection volume: 200
Detector: UV 270

CHROMATOGRAM
Retention time: 2.85

OTHER SUBSTANCES
Extracted: N-acetyl p-aminobenzoic acid

REFERENCE
Derewlany,L.O.; Knie,B.; Koren,G. Human placental transfer and metabolism of p-aminobenzoic acid, *J.Pharmacol.Exp.Ther.*, **1994**, *269*, 761–765.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80Ts (Tosoh Co., Japan)
Mobile phase: MeCN:water:acetic acid 10.5:89.5:1
Flow rate: 0.8
Injection volume: 20-30
Detector: UV 254

CHROMATOGRAM
Retention time: 11.7

OTHER SUBSTANCES
Simultaneous: carteolol

REFERENCE
Umehara,K.; Kudo,S.; Odomi,M. Involvement of CYP2D1 in the metabolism of carteolol by male rat liver microsomes, *Xenobiotica*, **1997**, *27*, 1121–1129.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm endcapped LichroCART RP18
Mobile phase: MeCN:7.5 mM pH 7.3 phosphate buffer 10:90
Column temperature: 40
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem II coulometric cell 5011, first electrode +450 mV, second electrode +630 mV; UV 254

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide

REFERENCE
Richter,K.; Oertel,R.; Kirch,W. New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 293–296.

SAMPLE
Matrix: solutions
Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483

g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES

Column: 100 × 4 10 μm Radial-Pak C18 (Waters)
Mobile phase: Gradient. MeOH:water from 30:70 to 70:30 over 30 min
Flow rate: 2
Injection volume: 5
Detector: F ex 370 em 450

CHROMATOGRAM

Retention time: 17
Limit of detection: 0.3-1 fmole

OTHER SUBSTANCES

Simultaneous: arachidic acid, arachidonic acid, benzoic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, salicylic acid, stearic acid, thymidine, uridine, valeric acid

KEY WORDS

derivatization

REFERENCE

Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *346*, 227–236.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer:triethylamine 4:4:92:0.01 (Buffer was 0.05% sodium octanesulfonate adjusted to pH 2.2 with 3 M phosphoric acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Simultaneous: benzaldehyde, benzoic acid, benzyl alcohol, protirelin

REFERENCE
Rao,G.N.; Sutherland,J.W.; Menon,G.N. High-performance liquid chromatographic assay for thyrotropin releasing hormone and benzyl alcohol in injectable formulation, *Pharm.Res.*, **1987**, *4*, 38–41.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Simultaneous: niacinamide, pyridoxal, pyridoxamine, thiamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a solution in MeCN:water 25:75.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 2 mL glacial acetic acid and 700 mg 1-octanesulfonic acid in 750 mL water.)
Flow rate: 1
Injection volume: 5
Detector: UV 285

CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Simultaneous: flucytosine

REFERENCE
Wintermeyer,S.M.; Nahata,M.C. Stability of flucytosine in an extemporaneously compounded oral liquid, *Antimicrob.Agents Chemother.*, **1996**, *40*, 407–409.

SAMPLE
Matrix: sunscreen
Sample preparation: Weigh out 1 g sunscreen, add 2-10 mL mobile phase, stir magnetically for 5 min, filter (0.45 μm Millex-HV), inject an aliquot.

HPLC VARIABLES
Column: 200 × 5 5 μm Nucleosil C18
Mobile phase: MeCN:15 mM phosphoric acid 3:97 (55:45 for simultaneous determination of PABA esters and benzocaine)
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Limit of detection: 500 ng/mL

REFERENCE
Bruze,M.; Gruvberger,B.; Thulin,I. PABA, benzocaine, and other PABA esters in sunscreens and after-sun products, *Photodermatol.Photoimmunol.Photomed.*, **1990**, *7*, 106–108.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 1 mL 1 mg/mL m-hydroxybenzoic acid in 8 M NaOH, heat at 120° for 1 h. Remove a 10 μL aliquot and add it to 990 μL 50 mM phosphoric acid, centrifuge at 12000 rpm (Beckman Microfuge B), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Yanapak ODS-T C18 (Yamagimoto)
Mobile phase: MeCN:200 mM pH 3.5 potassium phosphate buffer 5:35
Column temperature: 55
Flow rate: 0.7
Injection volume: 10
Detector: E, Yanaco Model VMD-101, glassy carbon electrode +1.1 V

CHROMATOGRAM
Retention time: 7.5
Internal standard: m-hydroxybenzoic acid (9.5)

OTHER SUBSTANCES
Noninterfering: furosemide, metoclopramide, sulfamethoxazole, diazepam, oxazolam, clonidine, hydralazine, osalmid

KEY WORDS
p-aminohippuric acid cleaved to p-aminobenzoic acid under these conditions

REFERENCE
Ito,S.; Maruta,K.; Imai,Y.; Kato,T.; Ito,M.; Nakajima,S.; Fujita,K.; Kurahashi,T. Urinary p-aminobenzoic acid determined in the pancreatic function test by liquid chromatography, with electrochemical detection, *Clin.Chem.*, **1982**, *28*, 323–326.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge, dilute 10-100 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:10 mM tetrabutylammonium chloride, pH 7.4 10:90
Column temperature: 40
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid, p-acetamidobenzoic acid, p-acetamidohippuric acid

REFERENCE
Karnes,H.T.; Riley,C.M.; Curry,S.H.; Schulman,S.G. Analysis of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (bentiromide) metabolites in urine by ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 377–388.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 50 μL 0.1 mM (sic) pH 5.0 sodium acetate buffer + 20 μL β-glucuronidase (Helix pomatia), heat at 37° for 3 h, add 20 μL glacial acetic acid, add 50 μL 1 mg/mL 3,5-diaminobenzoic acid in MeOH, add 50 μL mobile phase, vortex for 30 s, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb R, S5, ODS 2
Mobile phase: MeCN:buffer 3:97 (Buffer was 5 mM 1-heptanesulfonic acid in glacial acetic acid (Waters PIC-B7), pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 15
Internal standard: 3,5-diaminobenzoic acid (18)
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid, p-acetamidohippuric acid, p-acetamidobenzoic acid

REFERENCE
Chan,K.; Miners,J.O.; Birkett,D.J. Direct and simultaneous high-performance liquid chromatographic assay for the determination of p-aminobenzoic acid and its conjugates in human urine, *J.Chromatogr.*, **1988**, *426*, 103–109.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine diluted 1:10 + 50 μL MeOH + 100 μL 0.5 M HCl + 100 μL 0.1% sodium nitrite in water, vortex, let stand for 10 min, add 100 μL 2% ammonium sulfamate in water, let stand for 15 min, add 100 μL 0.05% 2-aminoanthracene

in MeCN (Caution! 2-Aminoanthracene causes cancer in experimental animals!), let stand for 15 min in the dark, add 5 mL diethyl ether, shake for 5 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 300 μL MeOH, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 5 μm YMC-Pack A-312 (YMC)
Mobile phase: MeOH:water:acetic acid 78:22:1
Flow rate: 1
Injection volume: 30
Detector: UV 279

CHROMATOGRAM

Retention time: 20
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: sulfanilamide, 4-aminobenzoyl-β-alanine

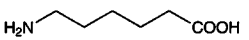
KEY WORDS

derivatization

REFERENCE

Hayashi,T.; Amino,M.; Uchida,G.; Sato,M. High-performance liquid chromatographic determination of primary aromatic amines in urine after derivatization to an azo dye with 2-aminoanthracene, *J.Chromatogr.B*, **1995**, *665*, 209–212.

Aminocaproic acid



Molecular formula: $C_6H_{13}NO_2$
Molecular weight: 131.17
CAS Registry No.: 60-32-2
Merck Index: 451

SAMPLE

Matrix: blood
Sample preparation: 20 μL Serum + 2 μL 3 mg/mL trans-4-aminomethylcyclohexanecarboxylic acid in water + 20 μL MeCN, mix, centrifuge at 10000 g for 3 min. Remove 5 μL of the supernatant and add it to 100 μL 25 mM pH 8 phosphate buffer, add 100 μL 300 μg/mL fluorescamine in acetone, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeCN:water:acetic acid:THF 30:69:0.5:0.5, containing 40 mM sodium acetate
Flow rate: 2
Injection volume: 20
Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 4
Internal standard: tranexamic acid (trans-4-aminomethylcyclohexanecarboxylic acid) (5)
Limit of detection: 6000 ng/mL

KEY WORDS

serum; for epsilon-aminocaproic acid; derivatization

REFERENCE

Lacroix,C.; Levert,P.; Laine,G.; Goulle,J.P. Microdosage de deux antifibrinolytiques (acide β-aminocaproïque et acide tranexamique) par chromatographie liquide et détection fluorimétrique [Microanalysis of two antifibrinolytics (epsilon-aminocaproic acid and tranexamic acid) by liquid chromatography and fluorometry], *J.Chromatogr.*, **1984**, *309*, 183–186.

SAMPLE

Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 10 μL 10% zinc sulfate, mix, add 100 μL MeOH, vortex, centrifuge for 1 min. Remove a 50 μL aliquot and add it to 300 μL IS solution, add 50 μL reagent, mix, after 1 min inject a 50 μL aliquot. (Prepare IS solution by adding 500 μL 1 mg/mL DL-valine to 20 mL 1 M pH 9.8 borate buffer. Prepare reagent by dissolving 20 mg o-phthalaldehyde and 24 mg N-acetyl-L-cysteine in 6 mL MeOH: water 50:50.)

HPLC VARIABLES

Column: 150 × 4.2 Nucleosil 5-C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 10 g/L (?) ammonium acetate containing 5 mM L-proline and 2.5 mM copper sulfate.)
Flow rate: 2
Injection volume: 50
Detector: F

CHROMATOGRAM

Retention time: 7

Internal standard: valine (9 (L), 13 (D))
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amino acids

KEY WORDS
derivatization; plasma

REFERENCE
Lam,S. High performance liquid chromatographic assay of Amicar, *epsilon*-aminocaproic acid, in plasma and urine after pre-column derivatization with *o*-phthalaldehyde for fluorescence detection, *Biomed.Chromatogr.*, **1990**, *4*, 175–177.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve an amount of formulation containing 10-20 mg dexpanthenol in 10 mL 0.5 M HCl, heat at 85 ± 2° for 30 min to hydrolyze dexpanthenol to aminopropanol. Remove an aliquot containing 1-2 mg dexpanthenol and add it to 10 mL 0.4 mg/mL fluorescamine in MeCN, add 2 mL ϵ-aminocaproic acid (concentration 60% of that of dexpanthenol) in mobile phase, make up to 25 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 Chromegabond C18
Mobile phase: MeOH:100 mM borate buffer adjusted to pH 8.0 ± 0.1 with 2 M NaOH 30:70
Flow rate: 1
Injection volume: 20
Detector: F ex 390 em 475-490 or UV 390

CHROMATOGRAM
Retention time: 12
Internal standard: ϵ-aminocaproic acid

OTHER SUBSTANCES
Simultaneous: dexpanthenol

KEY WORDS
ϵ-aminocaproic acid is IS; derivatization

REFERENCE
Umagat,H.; Tscherne,R. High performance liquid chromatographic determination of panthenol in bulk, premix, and multivitamin preparations, *Anal.Chem.*, **1980**, *52*, 1368–1370.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out powdered tablet containing aminocaproic acid, dissolve in 100 mL water, filter (0.45 μm). Mix a 5 mL aliquot of the filtrate with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. Injections, syrup. Weigh out amount of injection or syrup containing 250 mg aminocaproic acid, dilute with 100 mL water, dilute an aliquot 5-fold with water. Mix a 5 mL aliquot with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL

with acetone:water 50:50, mix, inject an aliquot. (Prepare buffer by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

HPLC VARIABLES

Guard column: C18 (Alltech)
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:water:acetic acid:triethylamine 60:38:1.5:0.5
Flow rate: 1.5
Injection volume: 20
Detector: UV 335

CHROMATOGRAM

Retention time: 4.5
Internal standard: tranexamic acid (6.5)

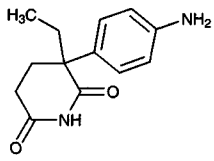
KEY WORDS

derivatization; tablets; injections; syrup

REFERENCE

Lau-Cam,C.A.; Roos,R.W. Assay of aminocaproic acid in dosage forms by reversed phase high performance liquid chromatography with dansylation, *J.Liq.Chromatogr.*, **1993**, *16*, 403–419.

Aminoglutethimide



Molecular formula: $C_{13}H_{16}N_2O_2$
Molecular weight: 232.28
CAS Registry No.: 125-84-8
Merck Index: 460
Lednicer No.: 1 257

SAMPLE

Matrix: blood, saliva, urine
Sample preparation: 300 μL Plasma, urine, or saliva + 150 μL 50 μg/mL IS in MeOH + 300 μL 100 mM pH 5.6 acetate buffer + 5 mL dichloromethane, vortex for 1 min, centrifuge at 1760 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeOH, vortex for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak Resolve silica (Waters)
Column: two Chiralcel OD cellulose tris(3,5-dimethylphenyl)carbamate columns in series
Mobile phase: Hexane:MeOH:isopropanol 65:17.5:17.5 0.7
Flow rate: 0.7
Injection volume: 50
Detector: UV 245

CHROMATOGRAM

Retention time: 32.65 (R), 43.66 (S)
Internal standard: (4'-aminophenyl)-3-methyl-1-methylpyrrolidine-2,5-dione (one enantiomer only) (37.74)
Limit of detection: 320 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: acetaminophen, carmustine, codeine, dexamethasone, hydrocortisone, ibuprofen, mitomycin, morphine, prednisone, tamoxifen, vincristine
Noninterfering: aspirin, busulfan, cyclophosphamide, indomethacin, methotrexate, vincristine

KEY WORDS

plasma; chiral

REFERENCE

Alshowaier,I.A.; el-Yazigi,A.; Ezzat,A.; El-Warith,A.E.; Nicholls,P.J. Liquid chromatographic separation and measurement of optical isomers of aminoglutethimide and its acetyl metabolite in plasma, saliva, and urine, *Ther.Drug Monit.*, **1995**, *17*, 538–543.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: EtOH:hexane 80:20
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: k' 2.00 ((R)-(+)), k' 5.14 ((S)-(-))

KEY WORDS
chiral

REFERENCE
Francotte,E.R.; Richert,P. Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs, *J.Chromatogr.A*, **1997**, *769*, 101–107.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount of finely powdered tablet corresponding to 20.83 mg aminoglutethimide, add 1 mL EtOH, add 5 mL water, sonicate for 30 min, make up to 10 mL with water, filter, discard the first 2 mL filtrate. Remove a 40 μL aliquot of the filtrate and add it to 500 μL pH 4.5 phosphate buffer, add 300 μL 2.6 mg/mL 1,2-naphthoquinone-4-sulfonic acid in water (prepare fresh each day), heat at 60° for 15 min, cool, add 4 mL n-butanol:ethyl acetate 2:1, shake vigorously for 30 s, centrifuge at 1750 rpm for 10 min. Remove a 100 μL aliquot of the organic layer and add it to 250 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 90:10
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.53
Limit of detection: 50 ng

KEY WORDS
derivatization; tablets

REFERENCE
Ozkirimli,S.; Sevingil,M. High pressure liquid chromatographic determination of aminoglutethimide, *Acta Pharm.Turc.*, **1989**, *31*, 57–60.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 25.00

KEY WORDS
narrow-bore; chiral; α 1.20

REFERENCE

Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 25
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 10.08, 11.05

KEY WORDS

chiral; comparison with capillary electrophoresis

REFERENCE

Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

SAMPLE

Matrix: urine
Sample preparation: 50 mL urine + 50 mL water, extract three times with 25 mL dichloromethane. Combine the organic layers and concentrate them under reduced pressure, evaporate to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OD + 250 × 4.6 10 μm Chiralcel OJ (Daicel)
Mobile phase: Hexane:isopropanol 50:50
Flow rate: 0.7
Detector: UV 257

CHROMATOGRAM

Retention time: 32.0 ((-)-S), 53.1 ((+)-R)

OTHER SUBSTANCES

Extracted: acetylaminoglutethimide

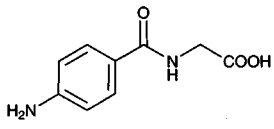
KEY WORDS

chiral

REFERENCE

Aboul-Enein,H.Y.; Islam,M.R. Direct enantiomeric high performance liquid chromatographic separation of aminoglutethimide and its major metabolite on a series of Chiralcel OD and Chiralcel OJ columns and its application to biological fluids, *Biomed.Chromatogr.*, **1991**, *5*, 74–77.

Aminohippuric acid



Molecular formula: $C_9H_{10}N_2O_3$
Molecular weight: 194.19
CAS Registry No.: 61-78-9, 94-16-6 (sodium salt)
Merck Index: 462

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or urine + 0.5-5 μg p-aminobenzoic acid + 200 μL MeCN, vortex for a few s, centrifuge at 800 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:0.04% pH 2.5 ± 0.05 phosphoric acid 3.5:96.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: p-aminobenzoic acid (8)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: iothalamate

KEY WORDS
plasma; dog; human; pharmacokinetics

REFERENCE
Prueksaritanont,T.; Chen,M.L.; Chiou,W.L. Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids, *J.Chromatogr.*, **1984**, *306*, 89–97.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 20 μg/mL p-aminobenzoic acid in MeCN, vortex for 15 s, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 10 μm μBondapak C18
Mobile phase: 67 mM pH 2.8 phosphate buffer containing 5 mM tetrabutylammonium phosphate (low-UV Pic A Reagent, Waters) (Buffer was KH_2PO_4:Na_2HPO_4 97.5:2.5.)
Flow rate: 2
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 7.4
Internal standard: p-aminobenzoic acid (13.0)
Limit of detection: 1000 ng/mL

KEY WORDS
serum

REFERENCE
Jenny,P.D.; Weber,A.; Smith,A.L. Quantitation of p-aminohippuric acid in biological fluids by high-performance liquid chromatography and dual-wavelength ultraviolet detection, *J.Chromatogr.*, **1989**, *490*, 213–218.

SAMPLE
Matrix: blood
Sample preparation: Add barbital to plasma. 100 μL Plasma + 500 μL MeOH, vortex for 15 s, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in buffer, inject a 15-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Octyl C8 (Rainin)
Mobile phase: MeOH:MeCN:buffer 90:10:300 (Buffer was 6.44 g KH_2PO_4, 7.04 g K_2HPO_4, and 14 mL 500 mM dodecyltriethylammonium phosphate (Regis) in 4 L water.)
Flow rate: 1
Injection volume: 15-20
Detector: UV 254

CHROMATOGRAM
Retention time: 12.0
Internal standard: barbital (15.9)
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Extracted: iothalamic acid

KEY WORDS
plasma

REFERENCE
Jayewardene,A.L.; Seneviratne,A.K.; Gambertoglio,J.G. Paired ion reversed-phase HPLC assay for the simultaneous determination of iothalamic acid and para aminohippuric acid in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 2395–2412.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 250 μg/mL acetaminophen in 100 mM HCl, add to SPE cartridge containing 150 mg 80-100 mesh Chromosorb P/NAW, elute with 1 mL ethyl acetate:MeOH 5:1, add the eluate to 50 μL 100 mM HCl, vortex for 15 s, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 5 μm C8
Mobile phase: MeCN:20 mM pH 3.3 phosphoric acid 2.5:97.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Internal standard: acetaminophen
Limit of detection: <1 μg/mL

OTHER SUBSTANCES
Extracted: iohexol

KEY WORDS
serum; SPE

REFERENCE
Andreeva,M.; Rapondjieva,A.; Deskova,D.; Tishkov,I.; Svinarov,D. Liquid chromatographic determination of iohexol and PAH with Chromosorb P column used for sample preparation (Abstract 175), *Ther.Drug Monit.*, **1995**, *17*, 427–427.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 150 μL salicylic acid in 1 M perchloric acid, centrifuge at 10000 g for 5 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Cosmosil MS-C18
Mobile phase: MeCN:water:glacial acetic acid 3:100:1, adjusted to pH 4.0 with 10 M NaOH
Column temperature: 40
Flow rate: 1.5
Injection volume: 30
Detector: F ex 270 em 350

CHROMATOGRAM
Retention time: 4.01
Internal standard: salicylic acid (17)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: p-acetamidobenzoic acid, p-acetamidohippuric acid, p-aminobenzoic acid

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Song,D.J.; Hsu,K.Y. Determination of p-aminobenzoic acid and its metabolites in rabbit plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1996**, *677*, 69–75.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix 100 μL plasma with 111 μg/mL 4-acetamidobenzoic acid in MeCN:water 10:90, vortex, add 25 μL 20% trichloroacetic acid, vortex, centrifuge at 10000 g for 10 min, inject a 25 μL aliquot. Urine. Mix 100 μL urine (diluted 1:50 with water) with 25 μL 333 μg/mL 4-acetamidobenzoic acid in MeCN:water 1:29, vortex, add 25 μL 20% trichloroacetic acid, vortex, centrifuge at 10 000 g for 10 min, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 8 × 4 5 μm Nucleosil 100 C18 AB ChromCart
Column: 125 × 4 5 μm Nucleosil 100 C18 AB ChromCart
Mobile phase: Gradient. MeOH:pH 3.9 buffer from 1:99 to 15:85 over 15 min. (pH 3.9 Buffer was 375 ml solution C containing 1.0 g sodium heptanesulfonate monohydrate and 2.7 mL 85% orthophosphoric acid made up to1000 mL with water. Solution C was 21.01 g citric acid monohydrate and 8.0 g sodium hydroxide in 1000 mL water.)
Flow rate: 1

Injection volume: 25
Detector: UV 273 for 5 min, UV 265 for 15 min

CHROMATOGRAM
Retention time: 3.6
Internal standard: 4-acetamidobenzoic acid (12.3)
Limit of detection: 200 ng/mL (plasma)
Limit of quantitation: 2.5 μg/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolite

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Decosterd,L.A.; Karagiannis,A.; Roulet,J.-M.; Bélaz,N.; Appenzeller,M.; Buclin,T.; Vogel,P.; Biollaz,J. High-performance liquid chromatography of the renal blood flow marker p-aminohippuric acid (PAH) and its metabolite N-acetyl PAH improves PAH clearance methods, *J.Chromatogr.B*, **1997**, *703*, 25–36.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 10-fold with water. Add 200 μL MeCN containing 20 μg/mL p-aminobenzoic acid to 100 μL diluted urine, vortex briefly, centrifuge at 12000 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 μm Ultrasphere C18
Column: 250 mm 5 μm Primesphere C18 (Torrance, USA)
Mobile phase: MeOH:buffer 18:82 (Buffer was 50 mM NaH_2PO_4 with 0.5 mM tetrabutyl ammonium hydrogen sulfate with an unadjusted pH of 4.11.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.2
Internal standard: p-aminobenzoic acid (9.8)

OTHER SUBSTANCES
Extracted: iothalamic acid

KEY WORDS
serum

REFERENCE
Agarwal,R. Chromatographic estimation of iothalamate and p-aminohippuric acid to measure glomerular filtration rate and effective renal plasma flow in humans, *J.Chromatogr.B*, **1998**, *705*, 3–9.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase. Urine.

Dilute 1:10 with water. Remove a 1 mL aliquot and add it to 1 mL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 10 × 3 anion-exchange guard column (Chrompack)
Column: 250 × 4.6 Partisil 10 SAX
Mobile phase: MeCN:25 mM pH 3 phosphate buffer 15:85
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: iothalamate
Noninterfering: acipimox, allopurinol, aspirin, atenolol, captopril, chlorthalidone, clonidine, digitoxin, digoxin, diltiazem, dipyridamole, enalapril, furosemide, gemfibrozil, hydralazine, hydrochlorothiazide, ibopamine, insulin, inulin, isosorbide dinitrate, α-methyldopa, nicardipine, nifedipine, prazosin, propranolol, salicylic acid, simvastatin, trinitrin, verapamil

KEY WORDS
plasma

REFERENCE
Gaspari,F.; Mainardi,L.; Ruggenenti,P.; Remuzzi,G. High-performance liquid chromatographic determination of iothalamic acid in human plasma and urine, *J.Chromatogr.*, **1991**, *570*, 435–440.

SAMPLE
Matrix: perfusate
Sample preparation: Adjust pH of 5-10 mL perfusate to 5 with 180 μL 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water containing 30 μL/L triethylamine, adjusted to pH 2.3 with phosphoric acid 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Extracted: procaine, aminobenzoic acid, 4-acetamidobenzoic acid

KEY WORDS
rabbit; chinchilla; pharmacokinetics

REFERENCE
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, *22*, 1357–1366.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge, dilute 10-100 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:10 mM tetrabutylammonium chloride, pH 7.4 10:90
Column temperature: 40
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 120 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, p-acetamidobenzoic acid, p-acetamidohippuric acid

REFERENCE
Karnes,H.T.; Riley,C.M.; Curry,S.H.; Schulman,S.G. Analysis of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (bentiromide) metabolites in urine by ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 377–388.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 50 μL 0.1 mM (sic) pH 5.0 sodium acetate buffer + 20 μL β-glucuronidase (Helix pomatia), heat at 37° for 3 h, add 20 μL glacial acetic acid, add 50 μL 1 mg/mL 3,5-diaminobenzoic acid in MeOH, add 50 μL mobile phase, vortex for 30 s, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb R, S5, ODS 2
Mobile phase: MeCN:buffer 3:97 (Buffer was 5 mM 1-heptanesulfonic acid in glacial acetic acid (Waters PIC-B7), pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 11.8
Internal standard: 3,5-diaminobenzoic acid (18)
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, p-acetamidohippuric acid, p-acetamidobenzoic acid

REFERENCE
Chan,K.; Miners,J.O.; Birkett,D.J. Direct and simultaneous high-performance liquid chromatographic assay for the determination of p-aminobenzoic acid and its conjugates in human urine, *J.Chromatogr.*, **1988**, *426*, 103–109.

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 1:100 or 1:500. 200 μL Diluted urine + 50 μL barbital solution, vortex for 15 s, inject a 20-30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere C8

Mobile phase: MeCN:MeOH:10 mM pH 7.5 potassium phosphate buffer:0.5 M dodecyl triethylammonium phosphate 6:94:300:0.6 (0.5 M Dodecyl triethylammonium phosphate was Q-12, Ion pair reagent, Regis Chemical Co.)

Flow rate: 1

Injection volume: 20-30

Detector: UV 254

CHROMATOGRAM

Retention time: 10.4

Internal standard: barbital (14.5)

Limit of quantitation: 75000 ng/mL

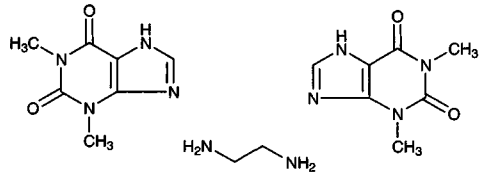
OTHER SUBSTANCES

Extracted: iothalamate

REFERENCE

Seneviratne,A.K.; Jayewardene,A.L.; Gambertoglio,J.G. Paired-ion reversed-phase HPLC assay for the determination of iothalamic acid and para aminohippuric acid in urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1311–1316.

Aminophylline



Molecular formula: $C_{16}H_{24}N_{10}O_4$
Molecular weight: 420.43
CAS Registry No.: 317-34-0
Merck Index: 485
Lednicer No.: 1 427

SAMPLE

Matrix: formulations
Sample preparation: Add 10 mL acetone:water 50:50 to 100-400 mg cream, stir for several min. Add 10 mL chloroform (Caution! Chloroform is a carcinogen!), shake for 2 min, centrifuge at 3000 rpm for 5 min, remove the organic layer. Mix a 1-5 mL aliquot of the organic layer with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 900 mg/mL sodium carbonate in acetone:water 50:50. Let the mixture stand at room temperature in the dark for 12 h. Make up to 50 mL. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb MV C18
Mobile phase: MeOH:water:acetic acid:triethylamine 69:29:1.5:0.5
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.01 (dansyl-theophylline), 9.21 (bis-dansyl-ethylenediamine)

KEY WORDS

derivatization; cream

REFERENCE

Haky,J.E.; Foss,W.M.; Marks,B.L. Analysis of aminophylline in thigh cream formulations by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2399–2414.

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Weigh out powdered tablets containing 100 mg aminophylline, add 50 mL water, sonicate for 15 min, make up to 100 mL with water, mix, filter. Remove a 5 mL aliquot of the filtrate and add it to 10 mL 5 mg/mL dansyl chloride in acetone and 5 mL buffer, mix gently, let stand in the dark for 12 h, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. Injections, oral liquids. Measure out an amount containing 100 mg aminophylline, make up to 100 mL with water, mix. Remove a 5 mL aliquot and add it to 10 mL 5 mg/mL dansyl chloride in acetone and 5 mL buffer, mix gently, let stand in the dark for 12 h, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. (Prepare buffer by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

HPLC VARIABLES

Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid:triethylamine 60:38:1.5:0.5 (A) or 65:33:1.5:0.5 (B)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 6.0 (theophylline (mobile phase B)), 7.3 (ethylenediamine (mobile phase B)), 7.45 (theophylline (mobile phase A)), 12.00 (ethylenediamine (mobile phase A))

KEY WORDS

derivatization; tablets; injections; oral solutions

REFERENCE

Lau-Cam,C.A.; Roos,R.W. Simultaneous high performance liquid chromatographic determination of theophylline and ethylenediamine in aminophylline dosage forms as their dansyl derivatives, *J.Liq.Chromatogr.*, **1991**, *14*, 1939–1956.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

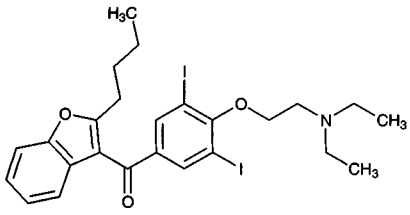
Also analyzed: acepromazine, acetaminophen, acetophenazine, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine,

phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Amiodarone



Molecular formula: $C_{25}H_{29}I_2NO_3$
Molecular weight: 645.32
CAS Registry No.: 1951-25-3
Merck Index: 504
Lednicer No.: 4 127, 156

SAMPLE

Matrix: blood
Sample preparation: Precipitate 100 µL serum with 200 µL 5 µg/mL IS in MeCN, centrifuge at 12 000 g for 5 min. Inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 µm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 µm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 10:90 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4.58
Internal standard: promazine (3.39)
Limit of detection: 90 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL cyano-bonded silica (J.T.Baker) SPE cartridge with 1 mL MeOH and three 1 mL portions of water. Mix 100 µL serum with 100 µL 2 µg/mL IS in MeOH:water 70:30 and 500 µL water. Add to the SPE cartridge, allow to pass through under gravity. Wash three times with 1 mL portions of water and with 1 mL MeOH:water 50:50. Elute with 1 mL MeOH containing 1 mL/L triethylamine. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase. Inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Zorbax cyano bonded silica
Mobile phase: MeCN:MeOH:phosphate buffer 23:37:40, adjusted to pH 3.5
Column temperature: 45
Flow rate: 0.8
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 7.92
Internal standard: tamoxifen (5.57)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: desethylamiodarone, bepridil, L8040 (Sanofi Recherche), trifluoperazine
Simultaneous: aprindine, bromocriptine, captopril, carbamazepine, chlorpromazine, diltiazem, dimefline, dipyridamole, disopyramide, flecainide, flurazepam, furosemide, imipramine, labetalol, miconazole, nifedipine, norverapamil, procainamide, propafenone, propranolol, quinidine, tocainide, trifluoropromazine, verapamil, warfarin

KEY WORDS
serum; SPE

REFERENCE
Pollak,P.T. A systematic review and critical comparison of internal standards for the routine liquid chromatographic assay of amiodarone and desethylamiodarone, *Ther.Drug Monit.*, **1996**, *18*, 168–178.

SAMPLE
Matrix: blood
Sample preparation: Mix 200 µL serum with 20 µL 25 µg/mL IS in MeOH and 100 µL 500 mM KH_2PO_4. Add 4 mL hexane, shake for 3 min, centrifuge, freeze at -20°. Evaporate the organic layer to dryness under a stream of argon in a 37° water bath, reconstitute in 100 µL MeOH, inject a 50 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 µm Supelguard LC-CN
Column: 150 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:MeOH:water: 500 mM KH_2PO_4 13.6:48:36:2.4
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6.7
Internal standard: L8040 (8.0)
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolite
Noninterfering: acebutolol, aprobarbital, atenolol, bupranolol, celiprolol, clobazam, debrisoquine, diazepam, diltiazem, flecainide, gallopamil, hexobarbiltal, lidocaine, mephenytion, metopolol, mexiletine, nadolol, pentobarbital, phenacetin, prazosin, procainamide, progesterone, propafenone, propranolol, quinidine, sotalol, theophylline, verapamil

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High performance liquid chromatographic analysis of some antiarrhythmic drugs in human serum using cyanopropyl derivatized silica phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1169–1181.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 8
Internal standard: minaprine (5.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: propafenone, desethylamiodarone, diltiazem, verapamil, nortriptyline, amitriptyline
Also analyzed: haloperidol, desipramine, imipramine, clomipramine

KEY WORDS

serum

REFERENCE

Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg Phenomenex C2 SPE cartridge (cat. no. AHO-0857) with 1 mL MeOH then 1 mL water (add analyte within 2-3 min). 500 μL Serum + 300 μL 2 M pH 4.5 sodium acetate + 10 μL 100 μg/mL triflupromazine in water, vortex for 5-10 s, add to SPE cartridge, wash with 1 mL water, 1 mL MeOH:water 1:1, 1 mL MeCN:water 1:1, elute with 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 Vydac C18 reverse phase (cat. no. 201SC)
Column: 150 × 4.6 5 μm UltraCarb 5 octadecylsilyl (cat. no. OOF-0351-EO)
Mobile phase: 875 mL MeOH:MeCN 1:1 + 125 mL 30 mM pH 4.0 ammonium acetate
Column temperature: 40
Flow rate: 1.5
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 6.5
Internal standard: triflupromazine (2.2)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, desipramine, digoxin, disopyramide, ethosuximide, flecainide, gentamicin, haloperidol, imipramine, kanamycin, li-

docvaine, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, propranolol, propoxyphene, quinidine, salicylic acid, streptomycin, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum; SPE

REFERENCE
Jandreski,M.A.; Vanderslice,W.E. Clinical measurement of serum amiodarone and desethylamiodarone by using solid-phase extraction followed by HPLC with a high-carbon reversed-phase column, *Clin.Chem.*, **1993**, *39*, 496–500.

SAMPLE
Matrix: blood
Sample preparation: Make serum alkaline with 500 mM KH_2PO_4, extract with hexane.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: MeCN:MeOH:water:500 mM KH_2PO_4 13.6:48:36:2.4
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High-performance liquid chromatographic determination of some antiarrhythmic drugs using cyanopropyl derivatized silica phase (Abstract 43), *Ther.Drug Monit.*, **1995**, *17*, 394–394.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL water + 500 μL 2 M pH 3.1 acetate buffer + 50 μL 32.4 μM IS in meOH + 6 mL dichloromethane, extract. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 4 μm Nova-Pak C18
Mobile phase: MeOH:water:concentrated (25%) ammonia 99.45:3:0.55
Flow rate: 1.1
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: L8040 (6)

OTHER SUBSTANCES
Extracted: metabolites, desethylamiodarone

KEY WORDS
serum; comparison with capillary electrophoresis

REFERENCE
Zhang,C.-X.; Aebi,Y.; Thormann,W. Microassay of amiodarone and desethylamiodarone in serum by capillary electrophoresis with head-column field-amplified sample stacking, *Clin.Chem.*, **1996**, *42*, 1805–1811.

SAMPLE
Matrix: blood, tissue
Sample preparation: Caution! Isopropyl ether may form explosive peroxides! Serum. 250 μL Serum + 100 μL 360 mM NaH_2PO_4 + 100 μL 6 μg/mL IS in MeOH + 200 μL isopropyl ether, vortex for 30 s, centrifuge at 3000 g for 3 min, inject a 50 μL aliquot of the organic layer. Tissue. Pound into a thin layer, freeze, lyophilize, pulverize, extract into isopropyl ether:MeOH 1:1, add IS, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve 5 μm spherical silica (Waters)
Mobile phase: MeOH:buffer 92:8 (Buffer was 2.2 g ammonium sulfate in 1 L water adjusted to pH 6.8 with 1 M NaOH.)
Flow rate: 1.8
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7
Internal standard: 2-ethyl-3-(3,5-dibromo-4γ-dipropylaminopropoxybenzoyl)benzothiophene (L8040) (4.4)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; normal phase; myocardium; lung; adipose; muscle

REFERENCE
Ou,C.-N.; Rognerud,C.L.; Duong,L.T.; Frawley,V.L. Liquid-chromatographic determination of amiodarone and N-desethylamiodarone in serum, *Clin.Chem.*, **1990**, *36*, 532–534.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 21.915

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: incubations
Sample preparation: 500 μL Incubation medium + 500 μL 100 mM pH 6.5 phosphate buffer + 5 mL hexane, shake for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness at 30° under a stream of nitrogen, reconstitute in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm Spherisorb CN
Mobile phase: Hexane:isopropanol:sulfuric acid 49.98:49.98:0.04
Flow rate: 1.5
Detector: UV 242

CHROMATOGRAM
Retention time: 8.8
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat; rabbit; normal phase; incubations

REFERENCE
Young,R.A.; Mehendale,H.M. In vitro metabolism of amiodarone by rabbit and rat liver and small intestine, *Drug Metab.Dispos.*, **1986**, *14*, 423–429.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 1.25 μg/mL IS in MeCN to microsomal incubation, mix vigorously for 30 s, centrifuge at 1500 g for 5 min, inject a 250 μL aliquot of the supernatant. (Use amber tubes.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil C18
Mobile phase: MeOH:water:58% ammonium hydroxide 88:10:2
Flow rate: 1.8
Injection volume: 250
Detector: UV 242

CHROMATOGRAM
Retention time: 8.1
Internal standard: 2-ethyl-3-(3,5-dibromo-4γ-dipropylaminopropoxybenzoyl)benzothiophene (L8040) (13.5)
Limit of detection: 50 nM

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat

REFERENCE
Trivier,J.M.; Pommery,J.; Libersa,C.; Caron,J.; Lhermitte,M. High-performance liquid chromatographic assay for amiodarone N-deethylation in microsomes of rat liver, *J.Chromatogr.*, **1992**, *579*, 269–276.

SAMPLE
Matrix: perfusate
Sample preparation: Centrifuge intestinal perfusate at 3000 rpm for 15 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μ Bondapak C18
Mobile phase: MeOH:10 mM pH 3.0 phosphoric acid 83:17
Flow rate: 2
Injection volume: 10
Detector: UV 242

KEY WORDS
rat; small intestine

REFERENCE
Martín-Algarra,R.V.; Pascual-Costa,R.M.; Merino,M.; Casabo,V.G. Intestinal absorption kinetics of amiodarone in rat small intestine, *Biopharm.Drug Dispos.*, **1997**, *18*, 523–532.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb
Mobile phase: MeCN:MeOH:buffer 3:6:1 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.9 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.46

OTHER SUBSTANCES
Simultaneous: flecainide acetate

REFERENCE
Paw,B.; Przyborowski,L.; Slawik,T. Determination of flecainide acetate in tablets by HPLC and UV-spectrophotometry, *Pharmazie*, **1998**, *53*, 97–98.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 200 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 2.50 phosphate buffer 15:80:5
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 6.2

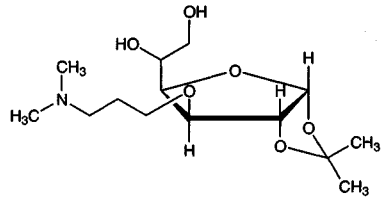
OTHER SUBSTANCES

Simultaneous: aprindine

REFERENCE

Misztal,G.; Przyborowski,L. Determination of aprindine in human plasma using reversed phase HPLC, *Pharmazie*, **1995**, *50*, 187–188.

Amiprilose



Molecular formula: $C_{14}H_{27}NO_6$
Molecular weight: 305.37
CAS Registry No.: 56824-20-5, 60414-06-4 (HCl)
Merck Index: 506

SAMPLE

Matrix: blood

Sample preparation: 250 μL Plasma + 100 μL 120 μg/mL IS in water, vortex for 1 min, add 200 μL 100 mM NaOH, add 6 mL dichloromethane, rotate for 15 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, add 75 μL 26.67 mg/mL 2-chloro-1-methylpyridinium iodide in MeCN, add 200 μL 2.5 mg/mL 1,8-naphthalic dicarboxylic acid in MeCN containing 6 μL/mL triethylamine, heat at 65° overnight, cool, inject a 20 μL aliquot. (Prepare 1,8-naphthalic dicarboxylic acid by hydrolyzing 1,8-naphthalic anhydride with 5% NaOH (cf. Org. Syn. 1973, Coll. Vol. V, 813).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS (Altex)
Mobile phase: MeOH:1 M ammonium acetate:N,N-dimethyloctylamine:water 65:2.5:0.03:32.5
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 340

CHROMATOGRAM

Retention time: 21 (main peak, a minor peak due to the other conformer is also seen)
Internal standard: 1,2-O-isopropylidene-3-O-[3'-(N,N-diisopropylamino)ethyl]-α-D-glucofuranose (28)
Limit of quantitation: 185 ng/mL

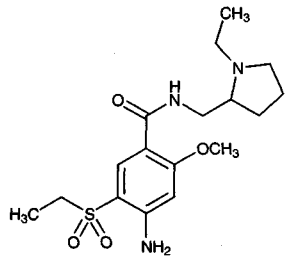
KEY WORDS

derivatization; plasma; pharmacokinetics

REFERENCE

Wu,S.T.; Benet,L.Z.; Lin,E.T. Determination of amprilose in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1997**, *692*, 149–156.

Amisulpride



Molecular formula: $C_{17}H_{27}N_3O_4S$
Molecular weight: 369.49
CAS Registry No.: 71675-85-9
Merck Index: 508

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 20-65 μL 10 μg/mL tiapride + 200 μL 1 M NaOH + 10 mL chloroform, shake for 30 min, centrifuge at 2000 g at -10° for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm RP-18 (Altex, ODS II, Beckman)
Mobile phase: MeOH:water:diethylamine 532:468:0.8
Flow rate: 1
Injection volume: 50
Detector: UV 226

CHROMATOGRAM

Retention time: 10.3
Internal standard: tiapride (6.4)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: sultopride
Simultaneous: sulpride, nitrazepam, flunitrazepam
Noninterfering: metoclopramide, oxazepam

KEY WORDS

plasma

REFERENCE

Bohbot,M.; Doare,L.; Diquet,B. Determination of a new benzamide, amisulpride, in human plasma by reversed-phase ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *416*, 414–419.

SAMPLE

Matrix: blood
Sample preparation: Add IS, extract with chloroform, back extract into an acidic medium, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:MeOH:buffer 200:90:100 (Buffer was 1% triethylamine adjusted to pH 3 with phosphoric acid.)
Flow rate: 1.3
Detector: F ex 280 em 370

CHROMATOGRAM

Retention time: 4.4

Internal standard: present but not specified (7.8)
Limit of detection: 0.25 ng/mL
Limit of quantitation: 0.5 ng/mL

KEY WORDS
plasma

REFERENCE
Moulin,A.; Truffer,D.; Rauch-Desanti,C.; Istin,M.; Grognet,J.-M.; Dufour,A. Comparison of HPLC and RIA methods applied to the quantification of amisulpride in human plasma, *Eur.J.Drug Metab.Pharmacokinet.*, **1991**, *Spec No.3*, 507–512.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 3.48
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifox-

amine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Isolute MF C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. 1 mL Plasma + 20 μL 10 μg/mL tiapride in MeOH + 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, wash with 80 μL MeOH, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES

Guard column: 20 × 4.6 40 μm Pelliguard Si
Column: 250 × 4.6 Chiralpak AS amylose carbamate (J.T. Baker)
Mobile phase: n-Hexane:EtOH:diethylamine 67:33:0.2
Column temperature: 25
Flow rate: 0.5
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 12 (S-(-)), 13 (R-(+))
Internal standard: tiapride (16)
Limit of quantitation: 2.5 ng/mL

KEY WORDS

mobile phase reservoir at 28°; plasma; chiral; SPE; pharmacokinetics

REFERENCE

Ascalone,V.; Ripamonti,; Malavasi,B. Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 95–105.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a 3 mL C8 SPE cartridge (Analytichem) with 2.7 mL MeOH and 2.7 mL buffer A, do not allow to dry. 1 mL Plasma + 100 μL in 10 mM

HCl + 1 mL buffer A, mix, add to the SPE cartridge, rinse tube with 1 mL buffer A and add to the SPE cartridge, wash with 2.7 mL water, wash with 2 mL buffer B, dry for 1 min, wash with 200 μL acetone, dry for 30 s, elute with 1 mL buffer C. Add 50 μL buffer D to the eluate, evaporate to dryness under a stream of air, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker quaternary amine-silicane-bonded ion-exchange SPE cartridge on top of a Baker carboxylic-acid bonded silica gel SPE cartridge and condition with 1 volume buffer D, 1 volume water, 1 volume MeOH, and 1 volume water. 1 mL Urine + 1 mL water + 100 μL alpiropride in 10 mM HCl, mix, add to the SPE cartridges, rinse tube with 2 mL water and add rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash with one volume water, wash with two volumes MeOH, dry for 1 min, elute with 1 mL buffer D. Evaporate the eluate to dryness under a stream of air at 45°, reconstitute the residue in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine and 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH.

HPLC VARIABLES

Guard column: 10 mm long reversed-phase pellicular (Chrompack)
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 16:8:76 (Buffer was 10 mL triethylamine in 760 mL water adjusted to pH 6.8 with acetic acid.)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM

Retention time: 4.9
Internal standard: alpiropride (6.0), amisulpride (4.9)

OTHER SUBSTANCES

Extracted: alizapride, metoclopramide
Simultaneous: acenocoumarol, acetaminophen, aspirin, caffeine, carbamazepine, clonazepam, codeine, isosorbide-5-mononitrate, nitrazepam, nitrofurantoin, theophylline
Noninterfering: amitriptyline, cisplatin, furosemide, indomethacin, isosorbide dinitrate, orphenadrine, propranolol

KEY WORDS

plasma; SPE; amisulpride is IS

REFERENCE

de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition a 3 mL C8 Analytichem SPE cartridge with 1 volume (2.7 mL) MeOH and 1 volume buffer A, do not allow to dry. Mix 1 mL plasma + 100 μL 10 μg/mL alpiropride in 10 mM HCl + 1 mL buffer A, add to SPE cartridge, rinse the sample container with 1 mL buffer A and add the rinse to the SPE cartridge, wash with 1 volume water, wash with 2 mL buffer B, dry the column for 1 min, wash with 200 μL acetone, dry for 30 s, elute with 1 mL buffer C, add 50 μL buffer D, evaporate to dryness under a stream of air, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker 3 mL ion exchange quaternary aminesilicane-bonded silica gel SPE cartridge on top of a 3 mL Baker carboxylic acid-bonded silica gel SPE cartridge, condition with 1 volume (2.7 mL) buffer D, 1 volume of water, 1 volume of MeOH, and 1 volume of water. Mix 1 mL urine + 100 μL 10 μg/mL alpiropride in 10

mM HCl + 1 mL water, add to SPE cartridges, rinse the sample container with 2 mL water and add the rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash the bottom column with 1 volume of water and 2 volumes of MeOH, dry the column for 1 min, elute with 1 mL buffer D, evaporate the eluate to dryness under a stream of air at 45°, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine + 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH (100 mM).)

HPLC VARIABLES
Guard column: 10 cm long Chrompack reverse-phase pellicular material
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 160:80:760 (Buffer was 10 mL triethylamine + 760 mL water adjusted to pH 6.8 with acetic acid (about 4.2 mL).)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM
Retention time: 4.9
Internal standard: alpiropride (6.0)

OTHER SUBSTANCES
Simultaneous: metoclopramide, alizapride, aspirin, theophylline, acetaminophen, caffeine, isosorbide-5-mononitrate, acenocoumarol, carbamazepine, nitrazepam, clonazepam
Noninterfering: indomethacin, orphenadrine, furosemide, cisplatin, amitriptyline, isosorbide dinitrate, propranolol
Interfering: codeine, nitrofurantoin

KEY WORDS
plasma; SPE; amisulpride is IS

REFERENCE
de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Isolute MF C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. Mix 1 mL urine, 40 μg metoclopramide, and 25 mL water. Mix 1 mL plasma with 20 μL 40 μg/mL metoclopramide in MeOH. Mix 1 mL diluted urine or plasma with IS with 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, wash with 80 μL MeOH, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pelliguard Si
Column: 250 × 4.6 Chiralpak AS amylose carbamate (J.T. Baker)
Mobile phase: n-Heptane:EtOH:diethylamine 70:29.8:0.2
Column temperature: 28
Flow rate: 0.5
Injection volume: 100
Detector: F ex 280 em 370

CHROMATOGRAM

Retention time: 14.8 (S-(-)), 17.0 (R-(+))
Internal standard: metoclopramide (11.5)
Limit of quantitation: 2.5 ng/mL (plasma), 50 ng/mL (urine)

KEY WORDS

mobile phase reservoir at 34°; plasma; chiral; SPE; pharmacokinetics

REFERENCE

Ascalone,V.; Ripamonti,; Malavasi,B. Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 95–105.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition a 100 mg C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. 1 mL Plasma + 20 μL 5 μg/mL IS in MeOH + 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40-45°, reconstitute the residue in 250 μL 25 mM pH 3 KH_2PO_4, inject a 150 μL aliquot. Alternatively, mix 1 g plasma, 20 μL 2.5 μg/mL IS in MeOH, 1 mL water, and 200 μL 1 M NaOH, add 7 mL diethyl ether:chloroform 95:5, shake in a tumble extractor at 40 rpm for 10 min, centrifuge at 500 g for 8 min, freeze at -20°. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeCN:25 mM pH 3 phosphate buffer 10:90, add 1 mL n-hexane shake in a tumble extractor at 20 rpm for 10 min, discard the hexane layer. Evaporate residual solvent under a stream of nitrogen at 40°, inject a 150 μL aliquot of the residual aqueous layer. Urine. Mix 1 mL urine and 20 μL 500 μg/mL IS in MeOH, dilute 100 fold with water. Mix 2 mL diluted urine and 200 μL 1 M NaOH, add 7 mL diethyl ether:chloroform 95:5, shake in a tumble extractor at 40 rpm for 10 min, centrifuge at 500 g for 8 min, freeze at -20°. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeCN:25 mM pH 3 phosphate buffer 10:90, add 1 mL n-hexane shake in a tumble extractor at 20 rpm for 10 min, discard the hexane layer. Evaporate residual solvent under a stream of nitrogen at 40°, inject a 50 μL aliquot of the residual aqueous layer. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES

Guard column: 20 × 4.6 40 μm Pelliguard LC8 (Supelco)
Column: 150 × 4.6 5 μm Hypersil C18 BDS
Mobile phase: MeCN:buffer 15:85 (Prepare buffer by mixing 25 mL 1 M KH_2PO_4, 950 mL water, and 1 mL triethylamine, adjust pH to 3 with phosphoric acid, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50-150
Detector: F ex 280 em 370

CHROMATOGRAM

Retention time: 4
Internal standard: L-(-)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-cyclopropylmethylsulfonyl-2-methoxybenzamide (Synthélabo Recherche) (7)
Limit of quantitation: 0.5 ng/mL, 100 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Malavasi,B.; Locatelli,M.; Ripamonti,M.; Ascalone,V. Determination of amisulpride, a new benzamide derivative, in human plasma and urine by liquid-liquid extraction or solid-phase extraction in combination with high-performance liquid chromatography and fluorescence detection. Application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 107–113.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 225.2

CHROMATOGRAM

Retention time: 8.923

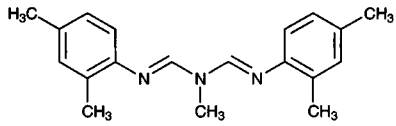
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Amitraz



Molecular formula: $C_{19}H_{23}N_3$
Molecular weight: 293.41
CAS Registry No.: 33089-61-1
Merck Index: 510
Lednicer No.: 4 36

SAMPLE

Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 313

CHROMATOGRAM

Retention time: 0.73

OTHER SUBSTANCES

Also analyzed: chlorpyrifos (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), permethrin (UV 229), phosmet (UV 229)

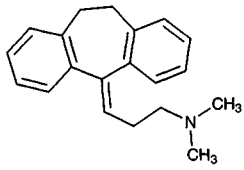
KEY WORDS

LOD 300 pg

REFERENCE

Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

Amitriptyline



Molecular formula: $C_{20}H_{23}N$
Molecular weight: 277.41
CAS Registry No.: 50-48-6, 549-18-8 (HCl)
Merck Index: 511
Lednicer No.: 1 151, 404

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 100 μL 400 μg/mL IS and 2 mL 500 mM NaOH, vortex briefly, add 4 mL heptane:isoamyl alcohol 98.5:1.5 and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL heptane:isoamyl alcohol 98.5:1.5 to extraction sample, mix. Combine the organic layers and extract them with 2 mL 50 mM sulfuric acid. Make the acid layer alkaline with 1 mL 1.0 M pH 9.0 carbonate/bicarbonate buffer and mix with 2 mL toluene:isoamyl alcohol 85:15 for 15 min. Evaporate the organic layer to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II OD
Mobile phase: MeCN:pH 3 phosphate buffer:n-nonylamine 40-50:60:0.12
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.48
Internal standard: doxepin (2.99)

OTHER SUBSTANCES

Extracted: nortriptyline

KEY WORDS

liver; lung; muscle; urine; pericardial fluid

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)

Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM
Retention time: 11.53
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites, clomipramine, desipramine, fluoxetine, imipramine maprotiline, nortriptyline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid
Interfering: levomepromazine

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 30 mg Oasis HLB SPE cartridge with 1 mL MeOH and 1 mL water. Acidify (?) mL serum with 20 μL phosphoric acid, vortex for 5 s, add to the SPE cartridge, wash with 1 mL MeOH :water 5:95, elute with 1 mL MeOH. Evaporate the eluate to dryness at 40° under a stream of nitrogen. Reconstitute the residue with 200 μL MeOH:20 mM pH 7 phosphate buffer 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 Sentry
Column: 150 × 3.9 5 μm Symmetry C18 (Waters)
Mobile phase: MeOH:20 mM pH 7 potassium phosphate 70:30
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 21
Internal standard: nordoxepin (4.9)

OTHER SUBSTANCES
Simultaneous: metabolites, doxepin, nortriptyline

KEY WORDS
pig; serum; SPE

REFERENCE
Cheng,Y.-F.; Phillips,D.J.; Neue,U.; Bean,L. Solid-phase extraction for the determination of tricyclic antidepressants in serum using a novel polymeric extraction sorbent, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2461–2473.

SAMPLE
Matrix: blood, gastric contents, tissue
Sample preparation: Blood. Mix 200 μL blood with 200 μL pH 11 borate buffer and 40 μL aqueous trimipramine solution. Extract with 800 μL hexane:2-butanol 98:2, centrifuge at 740 g for 10 min, evaporate the organic phase under vacuum at 40° (Buchler Vortex Evaporator, USA). Dissolve the residue in 100 μL mobile phase, inject an aliquot. Tissue, gastric contents. Homogenize tissue in pH 11 borate buffer to a final concentration 200 mg tissue/mL homogenate (Ultraturrax T5 homogenizer, IKA, Germany), dilute gastric contents 1:9 with water. Mix 200 μL tissue homogenate or diluted gastric contents with 40 μL aqueous trimipramine solution. Extract with 800 μL hexane:2-butanol 98:2, centrifuge at 740 g for 10 min, evaporate the organic phase under vacuum at 40° (Buchler Vortex Evaporator, USA). Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Buffer was saturated aqueous sodium tetraborate adjusted to pH 11 with 6 M NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-Si
Mobile phase: MeCN:25 mM ammonium acetate 90:10
Flow rate: 3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Internal standard: trimipramine maleate
Limit of detection: 40 nmol/kg (blood), 200 nmol/kg (tissue)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood; bone marrow; kidney; liver; lung; muscle; pharmacokinetics; pig; vitreous humor

REFERENCE
Hilberg,T.; Ripel,.; Smith,A.J.; Slordal,L.; Morland,J.; Bjorneboe,A. Postmortem amitriptyline pharmacokinetics in pigs after oral and intravenous routes of administration, *J.Forensic Sci.*, **1998**, *43*, 380–387.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Vortex 1 mL plasma or microsomal incubation with 200 μL 1 μg/mL desipramine and 100 μL 5 M NaOH for 10 s, add 5 mL butan-1-ol:hexane 2:98, vortex for 1 min, centrifuge at 2000 g and 4° for 5 min, evaporate the organic phase to dryness at 40° using a vacuum vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 30:70 (Buffer was water containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 50

Detector: UV 240

CHROMATOGRAM
Retention time: 10.5
Internal standard: desipramine (6.3)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: nortriptyline
Simultaneous: ketoconazole
Noninterfering: diazepam, furafylline, hydroxyamitriptyline, hydroxynortriptyline, quinidine, mephenytoin, triacetyloleandomycin

KEY WORDS
human; liver; rat; plasma

REFERENCE
Ghahramani,P.; Lennard,M.S. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *685*, 307–313.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15 000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 16.2 (A), 28.5 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES
Extracted: amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 15.878

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: serum
Sample preparation: 1 mL Serum + 500 μL 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μL IS in EtOH:water 50:50 + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Remove the organic layer and add it to 150 μL 22 mM pH 2.5 KH_2PO_4/phosphoric acid buffer, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Discard the organic layer, inject a 65 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 Supelco C18
Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH_2PO_4 containing 1.5 mL/L triethylamine, adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 65
Detector: UV 240

CHROMATOGRAM

Retention time: 11.5
Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)

OTHER SUBSTANCES

Extracted: citalopram, nortriptyline
Simultaneous: chlorprothixene, clomipramine, clozapine, flupenthixol, haloperidol, levomepromazine, perphenazine, zuclopenthixol
Noninterfering: benzodiazepines
Interfering: didesmethylclomipramine, levomepromazine

KEY WORDS

serum

REFERENCE

Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites, *J.Chromatogr.B*, **1996**, *675*, 83–88.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 239

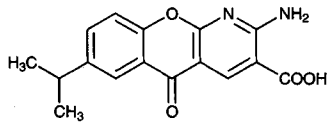
OTHER SUBSTANCES

Also analyzed: chlorpromazine, clomipramine, promazine, promethazine, thymol

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

Amlexanox



Molecular formula: $C_{16}H_{14}N_2O_4$
Molecular weight: 298.30
CAS Registry No.: 68302-57-8
Merck Index: 515

SAMPLE

Matrix: tissue
Sample preparation: Cut eye tissue in small pieces, add 5 mL MeoH, shake for 30 min, let stand overnight, centrifuge at 3000 rpm for 10 min. Remove 4 mL of the supernatant and evaporate it to dryness under vacuum, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: Schim-pack C18
Mobile phase: MeCN:50 mM pH 8.0 NaH_2PO_4 25:73
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 350 em 402

CHROMATOGRAM

Internal standard: 4-methylumbelliferone
Limit of detection: < 4000 ng/g

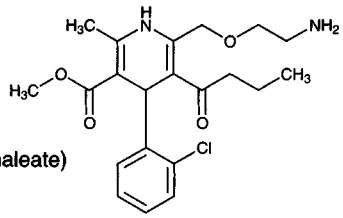
KEY WORDS

rat; eye

REFERENCE

Rankov,G.; Sasaki,K.; Fukuda,M. Pharmacodynamics of Amlexanox (AA-673) in normal and anaphylactic rat conjunctiva and its effect on histamine concentration, *Ophthalmic Res.*, **1990**, *22*, 359–364.

Amlodipine



Molecular formula: $C_{20}H_{25}ClN_2O_5$
Molecular weight: 408.88
CAS Registry No.: 88150-42-9, 111470-99-6 (besylate), 88150-47-4 (maleate)
Merck Index: 516
Lednicer No.: 4 108

SAMPLE

Matrix: blood

Sample preparation: Mix 5.0 mL plasma with 250 ng chloroamlodipine, add 250 μL 1 M sodium hydroxide and 5 mL chloroform, shake for 10 min, centrifuge at 3500 g. Dry the organic layer in a vacuum centrifuge, wash the tube twice with 500 μL chloroform and dry again. Dissolve the extract in 70 μL MeOH:pH 4.5 acetic buffer 60:40, inject a 50 μL aliquot. (For added sensitivity each eluting enantiomer may be trapped separately on 20 × 4.6 5 μm Supelcosil LC8 columns and eluted from these columns with MeCN:10 mM pH 4.5 acetate buffer 45:55 and chromatographed on 150 × 4.6 4 μm Symmetry C 8 columns.)

HPLC VARIABLES

Column: 150 × 4 Chiral AGP (ChromTech, Haegerstern, Sweden)
Mobile phase: 1-Propanol:10 mM pH 4.5 acetate buffer 1:99
Column temperature: 30
Flow rate: 0.9
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 11. 5 (R-(+)-), 15.4 (S-(-)-)
Internal standard: chloroamlodipine (29.2 (R-(+)), 36.6 (S-(-))

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Luksa,J.; Josic,D.; Kremser,M.; Kopitar,Z.; Milutinovic,S. Pharmacokinetic behaviour of R-(+)- and S-(-)-amlodipine after single enantiomer administration, *J.Chromatogr.B*, **1997**, *703*, 185–193.

SAMPLE

Matrix: blood

Sample preparation: 5 mL Plasma + 250 ng chloramlodipine + 250 μL 1 M NaOH + 5 mL chloroform, shake for 10 min, centrifuge at 3500 g. Evaporate the organic layer to dryness in a vacuum centrifuge, add 500 μL chloroform to the residue, evaporate to dryness, add 500 μL chloroform to the residue, evaporate to dryness, reconstitute with 70 μL MeOH:pH 4.5 acetate buffer 60:40, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A, collect each enantiomer on SEPARATE columns B. After 40 min elute the column B containing R-amlodipine with mobile phase B onto column C, monitor the effluent from column C, later elute the column B containing S-amlodipine with mobile phase B onto column C, monitor the effluent from column C.

HPLC VARIABLES

Column: A 150 × 4 Chiral AGP (ChromTech); B 20 × 4.6 Supelcosil LC-8; C 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: A n-Propanol:10 mM pH 4.5 acetate buffer 1:99; B MeCN:10 mM pH 4.5 acetate buffer 45:55

Column temperature: 30 (column C only)
Flow rate: 0.9
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 42.9 (R-(+)), 58.3 (S-(-)) [after start of analysis]
Internal standard: chloramlodipine (Lek)
Limit of detection: <5 ng/mL

KEY WORDS
chiral; plasma; column-switching

REFERENCE
Luksa,J.; Josic,D.; Podobnik,B.; Furlan,B.; Kremser,M. Semi-preparative chromatographic purification of the enantiomers of S-(-)-amlodipine and R-(+)-amlodipine, *J.Chromatogr.B*, **1997**, *693*, 367–375.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.093

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Inject an 800 μL aliquot of a 1.3 mg/mL solution.

HPLC VARIABLES
Column: 150 × 10 Chiral AGP (ChromTech, Sweden)

Mobile phase: n-Propanol:10 mM pH 4.5 acetate buffer 1:99
Flow rate: 4
Injection volume: 800
Detector: UV 240

CHROMATOGRAM

Retention time: 35 (R-(+)), 48 (S-(-))

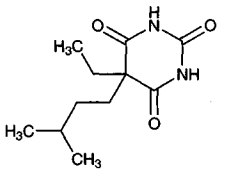
KEY WORDS

chiral; semi-preparative

REFERENCE

Luksa,J.; Josic,D.; Podobnik,B.; Furlan,B.; Kremser,M. Semi-preparative chromatographic purification of the enantiomers of S-(-)-amlodipine and R-(+)-amlodipine, *J.Chromatogr.B*, **1997**, *693*, 367–375.

Amobarbital



Molecular formula: $C_{11}H_{18}N_2O_3$
Molecular weight: 226.28
CAS Registry No.: 57-43-2, 64-43-7 (sodium salt)
Merck Index: 607
Lednicer No.: 1 268

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 23.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)

Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 6.05
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 µL plasma then 50 µL 10 µg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 µL mobile phase, inject a 15 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 9.64
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.617

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk quantities. Dissolve 25 mg bulk quantities in 25 mL MeCN, dilute 1:10 with MeCN, inject an aliquot. Tablets. Crush tablet, add 46 mg to 10 mL MeCN, shake for 24 h, dilute 1 mL clear supernatant 1:10 with MeCN, inject an aliquot. Capsules. Powder capsule, add 35 mg to 1 mL 10% sulfuric acid, shake, make up to 10 mL with MeCN, shake for 24 h, dilute 1 mL clear supernatant 1:10 with MeCN, inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 2 37-53 μm Whatman pellicular ODS
Column: 250 × 4.6 5 μm Econosphere C18 (Alltech)
Mobile phase: MeCN:water 30:70
Flow rate: 1.2
Detector: UV 198

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
tablets; capsules

REFERENCE
Soine,W.H.; Graham,R.M.; Soine,P.J. Identification of 5-ethyl-5-(2-methylbutyl)barbituric acid as an impurity of manufacture in amobarbital, *J.Pharm.Sci.*, **1992**, *81*, 362–364.

SAMPLE
Matrix: saliva
Sample preparation: Saliva sample obtained by chewing on 100 sq. cm Parafilm, centrifuge at 3000 g for 5 min. Remove a 1 mL aliquot of the supernatant and add it to 100 μL 5 μg/mL hexobarbital solution, add the mixture to a 3 mL octadecyl Baker-10 SPE cartridge, wash with three 3 mL portions of water, elute with 800 μL MeOH, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 30 × 4 5 μm Develosil ODS-5 (Nomura)
Column: 150 × 4 5 μm Develosil ODS-5 (Nomura)
Mobile phase: MeOH:water 50:50
Flow rate: 0.8
Injection volume: 50
Detector: UV 240 following a 150 × 3 sulfonated hollow-fiber membrane reactor (Dionex AFS-2) which was surrounded by 50 mM pH 10.2 ammonium hydroxide solution

CHROMATOGRAM
Retention time: 14
Internal standard: hexobarbital (10)
Limit of detection: 0.5-2.5 ng

OTHER SUBSTANCES
Simultaneous: barbital, phenobarbital

KEY WORDS
SPE; post-column reaction

REFERENCE
Haginaka,J.; Wakai,J. Liquid chromatographic determination of barbiturates using a hollow-fibre membrane for postcolumn pH modification, *J.Chromatogr.*, **1987**, *390*, 421–428.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 8
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: barbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, pentobarbital, phenobarbital, secobarbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate a solution in water, MeOH, or diethyl ether to dryness, add a 3-fold molar excess of triethylamine, add 0.5-3 mL MeCN, add a 3-fold molar excess of N-chloromethyl-4-nitrophthalimide, heat at 60° for 1 h, inject an aliquot. (Preparation of N-chloromethyl-4-nitrophthalimide is as follows. Suspend 130 g 4-nitrophthalimide in 80 mL 40% formaldehyde solution, add 200 mL water, reflux for 4 h, filter while hot, N-(hydroxymethyl-4-nitrophthalimide crystallizes on cooling (cf. J. Am. Chem. Soc. 1922, 44, 817). Mix a suspension of 2.26 g N-hydroxymethyl-4-nitrophthalimide in 10-15 mL ether with a suspension of 2.1 g phosphorus pentachloride in 10-15 mL ether, after 10 min heat on a water bath, cool in an ice-salt mixture, add ice-water dropwise with shaking, filter to obtain N-chloromethyl-4-nitrophthalimide, dry under vacuum (cf. Chem. Ber. 1959, 9, 1258).)

HPLC VARIABLES
Column: 7 μm LiChrosorb RP8
Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 4 ng

OTHER SUBSTANCES
Extracted: secobarbital
Simultaneous: cyclobarbital, methylphenobarbital, phenobarbital

KEY WORDS
derivatization

REFERENCE
Lindner,W.; Santi,W. N-chloromethylphthalimides as derivatization reagents for high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *176*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methabarbital, methohexital, phenobarbital, phenytoin, secobarbital, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 1.95

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, butabarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 2 μBondapak C18
Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid
Flow rate: 0.27
Injection volume: 5
Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Extracted: butethal, butabarbital, talbutal, butalbital, pentobarbital

KEY WORDS
mass spectra given

REFERENCE
Ryan,T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J.Liq.Chromatogr.*, **1994**, *17*, 867–881.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.94 (A), 5.59 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, pheno-

barbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 200 μL Urine + 1 mL saturated ammonium sulfate, extract with 2 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

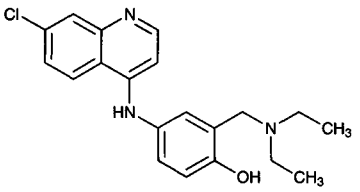
HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltech C18
Mobile phase: MeCN:25 mM pH 6.5 sodium phosphate buffer 20:80
Flow rate: 1.4
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 41.5

OTHER SUBSTANCES
Extracted: metabolites, conjugates

REFERENCE
Soine,W.H.; Soine,P.J.; Overton,B.W.; Garrettson,L.K. Product enantioselectivity in the N-glucosylation of amobarbital, *Drug Metab.Dispos.*, **1986**, *14*, 619–621.

Amodiaquin



Molecular formula: $C_{20}H_{22}ClN_3O$
Molecular weight: 355.87
CAS Registry No.: 86-42-0, 6398-98-7 (dihydrochloride dihydrate)
Merck Index: 609
Lednicer No.: 4 140

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 4.62
Limit of detection: <120 ng/mL

KEY WORDS

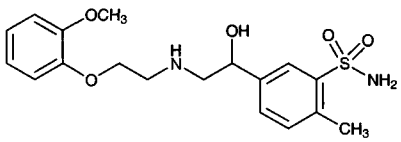
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Amosulalol



Molecular formula: $C_{18}H_{24}N_2O_5S$
Molecular weight: 380.47
CAS Registry No.: 85320-68-9
Merck Index: 614

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL saturated sodium bicarbonate solution and 100 μL water to 1.5 mL plasma, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 2.5 mL 400 mM HCl. Shake, centrifuge, and discard the organic layer. Add 2 mL saturated sodium bicarbonate solution to the aqueous layer and extract again with 5 mL ethyl acetate. Evaporate the organic layer to dryness under reduced pressure, reconstitute the residue with 50 μL 100 mM sodium bicarbonate. Add 100 μL 5 mg/mL dansyl chloride in acetone. Heat at 35° for 90 min. Add 5 mL distilled water and extract with 5 mL diethyl ether. Evaporate the organic layer to dryness at 45°, reconstitute the residue with 60 μL mobile phase. Inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 Nucleosil SI100-5 (Chemco, Japan)
Mobile phase: MeOH:benzene 1:100 (Caution! Benzene is a carcinogen!)
Injection volume: 20-50
Detector: F ex 352 em 500

KEY WORDS

amosulalol is IS; plasma; human; rat; dog; normal phase

REFERENCE

Matsushima,H.; Kamimura,H.; Soeishi,Y.; Watanabe,T.; Higuchi,S.; Tsunoo,M. Pharmacokinetics and plasma protein binding of tamsulosin hydrochloride in rats, dogs, and humans, *Drug Metab.Dispos.*, **1998**, *26*, 240–245.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL 0.5 μg/mL IS in water + 0.5 g sodium bicarbonate + 4 mL ethyl acetate, extract, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL 3 mg/mL sodium bicarbonate in water and 200 μL 500 μg/mL 5-di-n-butylaminonaphthalene-1-sulfonyl chloride (Bans-Cl) in acetone, heat at 45° for 90 min, cool, add 4 mL diethyl ether, wash mixture with 3 mL water for 10 s. Remove the organic layer and evaporate it to dryness at 40-50°, reconstitute the residue in 100 μL benzene (CAUTION! Benzene is a carcinogen!), inject a 5-10 μL aliquot. (Derivatization can also be performed with 5-dimethylaminonaphthalene-1-sulfonyl chloride (Dans-Cl), F ex 250 em 505, retention time of derivative 4.1 min.)

HPLC VARIABLES

Column: 150 × 4 5 μm LiChrosorb SI-60
Mobile phase: Benzene:MeOH 50:1 (CAUTION! Benzene is a carcinogen)
Flow rate: 2
Injection volume: 5-10
Detector: F ex 356 em 500

CHROMATOGRAM

Retention time: 2.9

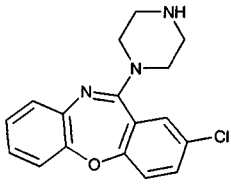
Internal standard: 5-[1-hydroxy-2-[2-(o-methoxyphenoxy)ethylamino]ethyl]-2-methoxybenzenesulfonamide (4.0)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; normal phase; derivatization; dog; pharmacokinetics

REFERENCE

Kamimura,H.; Sasaki,H.; Kawamura,S. Determination of the α,β-adrenoceptor blocker YM-09538 in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1981**, *225*, 115–121.

Amoxapine



Molecular formula: $C_{17}H_{16}ClN_3O$
Molecular weight: 313.79
CAS Registry No.: 14028-44-5
Merck Index: 616
Lednicer No.: 2 428

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 100 mM HCl + 400 µL 1 M NaOH + 6 mL ethyl acetate, shake for 15 min, centrifuge at 4000 g for 10 min. Transfer top organic layer to another tube and re-extract the analyte with 3 mL 50 mM HCl. Evaporate organic layer in water bath under nitrogen at 45°. Dissolve the residue in 120 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: Perisorb RP-8 (Upchurch)
Column: 100 × 4.6 3 µm Supelco C8-DB
Mobile phase: MeCN:MeOH:buffer 20:18:62 (Buffer was 50 mM sodium monobasic phosphate, contains 2.5 mL/L triethylamine, pH adjusted to 2.7 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 30
Detector: UV 230

CHROMATOGRAM

Retention time: 14.3
Internal standard: amoxapine

OTHER SUBSTANCES

Extracted: metabolites, clozapine
Simultaneous: amitryptiline, atenolol, bupropion, cogentin, desipramine, diazepam, doxepin, fluoxetine, haloperidol, imipramine, loxapine, medazepam, nortryptiline, oxazepam, paroxetine, phenytoin, propranolol, sertraline, thiothixene, trazadone, trifluoperazine, valproic acid, verapamil
Interfering: carbamazepine, desmethylsertraline

KEY WORDS

serum; amoxapine is IS

REFERENCE

Hariharan,U.; Hariharan,M.; Naickar,J.S.; Tandon,R. Determination of clozapine and its two major metabolites in human serum by liquid chromatography using ultraviolet detection, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 2409–2417.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 µL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 µL aliquot of the aqueous phase (J.Liq.Chromatogr. 1981, 4, 849).

HPLC VARIABLES

Guard column: 23 × 3.9 Bondapak/Corasil C 18

Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: clomipramine (8.5)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amoxapine, amitriptyline, chlordiazepoxide, chlorpromazine, cimetidine, desipramine, diazepam, doxepin, flurazepam, imipramine, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine
Interfering: nortriptyline

KEY WORDS
plasma

REFERENCE
Wong,S.H.Y.; Waugh,S.W. Determination of the antidepressants maprotiline and amoxapine, and their metabolites, in plasma by liquid chromatography, *Clin.Chem.*, **1983**, *29*, 314–318.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, clomipramine, doxepin, desipramine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE

Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL 2-hydroxydesmethylimipramine, mix, add 300 μL 2 M pH 9.7 carbonate buffer, mix, add 4 mL heptane:isopentyl alcohol 93:7, shake mechanically for 20 min, centrifuge at 1600 g for 5 min. Remove the organic layer and add it to 250 μL 7 mM orthophosphoric acid, vortex vigorously, centrifuge at 1600 g for 10 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb C6

Mobile phase: 105 μM nonylamine in MeCN:5 mM KH_2PO_4 + 14 mM orthophosphoric acid 23:77

Column temperature: 35

Flow rate: 2.2

Injection volume: 100

Detector: UV 210

CHROMATOGRAM

Retention time: 22

Internal standard: 2-hydroxydesmethylimipramine

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, loxapine

Simultaneous: propranolol, doxepin, desmethylimipramine, haloperidol, protriptyline, imipramine, amitriptyline

Noninterfering: chlorpromazine, clomipramine, maprotiline, nortriptyline, thioridazine, trimipramine, trifluoperazine

KEY WORDS

plasma

REFERENCE

Cheung,S.W.; Tang,S.W.; Remington,G. Simultaneous quantitation of loxapine, amoxapine and their 7- and 8-hydroxy metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *564*, 213–221.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 μg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 μL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 11.18
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, propranolol, clovoxamine, fluvoxamine, fenfluramine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 75 μL MeCN containing 4 mg/mL loxapine + 2 mL 250 mM NaOH + 400 μL isoamyl alcohol, vortex vigorously, let stand for 5 min, add 10 mL heptane, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Remove the upper heptane layer and add it to 1 mL 100 mM pH 3 glycylglycine buffer, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Discard the heptane layer, add 1 mL 250 mM NaOH to the aqueous layer, add 5 mL n-pentane, shake for 1 h, centrifuge at 2000 g for 30 min. Remove the organic layer and evaporate it to dryness under educed pressure, reconstitute the residue in 70 μL mobile phase, vortex vigorously, centrifuge at 2000 g for 2-3 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 40 μm pellicular silica (CEL Associates, Houston)
Column: 100 × 6 3 μm silica 80 Å (CEL Associates, Houston)
Mobile phase: MeCN:buffer 20:80 containing 21 mM n-nonylamine, pH 7.4-7.8 (Buffer was 25 mM Na_2HPO_4 adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1.6
Injection volume: 50
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 9.63
Internal standard: loxapine (22.68)

OTHER SUBSTANCES
Extracted: doxepin
Simultaneous: amitriptyline, chlorpromazine, clomipramine, desipramine, fluoxetine, imipramine, mianserin, nortriptyline, thioridazine, trimipramine
Noninterfering: diazepam

KEY WORDS
serum; amoxapine is IS

REFERENCE
Adamczyk,M.; Fishpaugh,J.R.; Harrington,C. Quantitative determination of *E*- and *Z*-doxepin and *E*- and *Z*-desmethyldoxepin by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 371–376.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 299

CHROMATOGRAM
Retention time: 7.57
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; tri-

fluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 6.61
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: fluoxetine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15 000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)

Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40

Flow rate: 0.6

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5.6 (A), 7.8 (B)

Internal standard: diazepam (4.4, A)

Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, dothiepin, doxepin, imipramine, melitracen, mianserin, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

Interfering: desipramine, maprotiline

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 14.187

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: < 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, carbamazepine, imipramine, nortriptyline
Also analyzed: doxepin, desipramine, protriptyline, cyclobenzaprine, maprotiline

KEY WORDS
UV spectra given

REFERENCE
Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.40 (A), 5.55 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

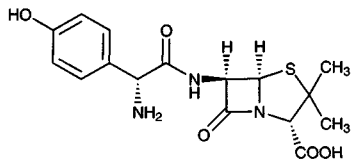
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Amoxicillin



Molecular formula: $C_{16}H_{19}N_3O_5S$
Molecular weight: 365.41
CAS Registry No.: 26787-78-0 (anhydrous), 61336-70-7 (trihydrate)
Lednicer No.: 4 179, 180

SAMPLE

Matrix: blood
Sample preparation: Vortex plasma sample for 15 s, centrifuge at 4000 rpm for 10 min at 4 °. 250 µL Plasma + 250 µL 5 µg/mL cefadroxil in 100 mM pH 2.5 KH_2PO_4, centrifuge at 4800 g for 30 min. Inject a 150 µL aliquot of the clear supernatant onto column A and elute to waste with mobile phase B, after 15 min elute the contents of column A onto column B with mobile phase A, after 5 min remove column A from the circuit, elute column B with mobile phase A, monitor effluent from column B. Re-equilibrate column A with mobile phase B.

HPLC VARIABLES

Column: A 40 × 4.6 10 µm Nucleosil 100 C18; B 250 × 4.6 5 µm Spherisorb ODS II
Mobile phase: A MeOH:10 mM sodium heptanesulfonate and 30 mM NaH_2PO_4 25:75 adjusted to pH 2.5 with phosphoric acid; B MeOH:10 mM sodium heptanesulfonate and 30 mM NaH_2PO_4 8:92 adjusted to pH 2.5 with phosphoric acid
Flow rate: 1.5
Injection volume: 150
Detector: UV 230

CHROMATOGRAM

Retention time: 34.8-38.8
Internal standard: cefadroxil (31.8-32.8)
Limit of quantitation: 50.1 ng/mL

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Muth,P.; Metz,R.; Beck,H.; Bolten,W.W.; Vergin,H. Improved high-performance liquid chromatographic determination of amoxicillin in human plasma by means of column switching, *J.Chromatogr.A*, **1996**, *729*, 259–266.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.067

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 12.5 min), evaporate to <1 mL under reduced pressure, add 200 μL 10 mM KH_2PO_4 containing 10 mM phosphoric acid and 10 mM sodium decanesulfonate, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 32:68 (Buffer was 15 mM phosphoric acid containing 7.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through

the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM

Retention time: 25
Internal standard: penicillin V (28.5)
Limit of detection: 1.4 ng/mL
Limit of quantitation: 2.0 ng/mL

OTHER SUBSTANCES

Extracted: ampicillin, cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS

derivatization; cow; SPE

REFERENCE

Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE

Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 10:90 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1

Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 6.7
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:10 mM pH 6.1 potassium phosphate buffer 5:95
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions
Sample preparation: Prepare amoxicillin sodium solutions in water adjusted to pH 4 with trifluoroacetic acid.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil ABZ+plus (Supelco)
Mobile phase: Gradient. A MeCN:0.1% pH 2.1 trifluoroacetic acid 7:93; B MeCN:0.1% pH 2.1 trifluoroacetic acid 20:80. A:B 100:0 for 13 min, from 100:0 to 40:60 in 2 min, maintain at 40:60 for 30 min, from 40:60 to 100:0 in 2 min, maintain at 100:0 for 13 min
Flow rate: 1
Injection volume: 5
Detector: UV 230; MS, PE-Sciex API I, ionspray interface at 5500 V, orifice potential voltage 50 V, m/z 100-800

CHROMATOGRAM
Retention time: 9.99

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Valvo,L.; Ciranni,E.; Alimenti,R.; Alimonti,S.; Draisci,R.; Giannetti,L.; Lucentini,L. Development of a simple liquid chromatographic method with UV and mass spectrometric detection for the separation of substances related to amoxicillin sodium, *J.Chromatogr.A*, **1998**, *797*, 311–316.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.85

OTHER SUBSTANCES

Also analyzed: antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 500 mg Isolute SCX SPE cartridge (Jones Chromatography, Hengoed, UK) with MeOH and water. Condition a 100 mg PGC (porous graphitic carbon) SPE cartridge (Shandon, Runcorn, UK) with acetone and pH 7.7 borate buffer. Add 20 mL water to 5 g tissue, homogenize, add 5 mL 170 mM sulfuric acid and 5 mL 5% aqueous sodium tungstate, mix well, centrifuge at 14000 g for 5 min. Discard pellet, add six drops orthophosphoric acid to the supernatant to adjust the pH to 2-2.5. Add it to the SCX SPE cartridge, allow to flow through the cartridge under vacuum at 2 mL/min, wash with 5 mL 10 mM sulfuric acid, elute with 10 mL pH 7.7 borate buffer. Add the eluate to the PGC SPE cartridge. Wash with 5 mL water, place in-line filter (0.2 μm, Anotop) below cartridge, elute with 20 mL acetone. Evaporate to dryness. Add 500 μL water to the dry residue, add 20 μL 2% acetic anhydride in MeCN and let stand for 3 min. Add 500 μL triazole/mercuric chloride derivatizing reagent and heat the mixture at 65° for 20 min. Inject a 100 μL aliquot. (Borate buffer was 200 mM boric acid adjusted to pH 7.7 with 40% NaOH solution. Triazole/mercuric chloride reagent was prepared by mixing 34.45 g 1,2,4-triazole with 150 mL water and 25 mL 10 mM mercuric chloride, adjusted to pH 9.0 with 1 M NaOH and made up to 250 mL with water.) GL –5 μm Kromasil KR 100 C8 (Hichrom)

HPLC VARIABLES

Column: 250 × 3.2 5 μm Kromasil KR 100 C8 (Hichrom)
Mobile phase: MeCN:buffer 20:80 (Buffer was 15 mM potassium dihydrogen phosphate and 15 mM sodium thiosulfate)
Flow rate: 0.55
Injection volume: 100
Detector: UV 325

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 5 μg/kg (muscle)
Limit of quantitation: 50 μg/kg (muscle), 100 μg/kg (liver)

OTHER SUBSTANCES
Extracted: ampicillin

KEY WORDS
SPE; cow; liver; muscle; derivatization

REFERENCE
Rose,M.D.; Tarbin,J.; Farrington,W.H.; Shearer,G. Determination of penicillins in animal tissues at trace residue concentrations: II. Determination of amoxicillin and ampicillin in liver and muscle using cation exchange and porous graphitic carbon solid phase extraction and high-performance liquid chromatography, *Food Addit.Contam.*, **1997**, *14*, 127–133.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak C18 SPE cartridge with 5 mL MeOH, 2 mL water, and 2 mL 2% trichloroacetic acid. Homogenize (Ultra-Turrax T25) 5 g blended tissue with 20 mL 10 mM pH 4.5 phosphate buffer at 10000 rpm for 1.5 min, centrifuge at 4500 rpm for 10 min, decant supernatant, homogenize residue with another 20 mL buffer, centrifuge. Combine the supernatants and filter them through glass wool, add 1 mL 75% trichloroacetic acid to the filtrate, vortex for 30 s, centrifuge at 4500 rpm for 20 min, filter the supernatant through glass wool. Add the filtrate to the SPE cartridge at 1-2 mL/min, wash with 2 mL 2% trichloroacetic acid, wash with 2 mL water, elute with 1.5 mL MeCN at 0.7 mL/min. Add the eluate to 500 μL water and 3 mL ethyl ether, vortex gently for 30 s, centrifuge at 2000 rpm for 3 min, discard the organic layer. Add 200 μL 20% trichloroacetic acid solution to the aqueous phase, vortex for 15 s, add 200 μL 7% formaldehyde in 400 mM citric acid, vortex for 30 s, heat in boiling water bath for 30 min, cool to room temperature, add 500 mg NaCl, mix briefly, add 3 mL ethyl ether, vortex for 1 min, centrifuge at 2000 rpm for 3 min, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, vortex thoroughly, inject a 50 μL aliquot.

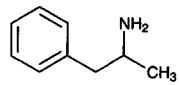
HPLC VARIABLES
Column: 250 × 4.6 S5 ODS2
Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM KH_2PO_4 adjusted to pH 5.6 with KOH.)
Flow rate: 1 for 10 min then 2
Injection volume: 50
Detector: F ex 358 em 440

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.5 ppb (catfish), 0.8 ppb (salmon)
Limit of quantitation: 1.2 ppb (catfish), 2.0 ppb (salmon)

KEY WORDS
derivatization; fish; catfish; salmon; SPE

REFERENCE
Ang,C.Y.W.; Luo,W.; Hansen,E.B.,Jr.; Freeman,J.P.; Thompson,H.C.,Jr. Determination of amoxicillin in catfish and salmon tissues by liquid chromatography with precolumn formaldehyde derivatization, *J.AOAC Int.*, **1996**, *79*, 389–396.

Amphetamine



Molecular formula: $C_9H_{13}N$
Molecular weight: 135.21
CAS Registry No.: 300-62-9, 139-10-6 (phosphate), 60-13-9 (sulfate), 1407-85-8 (d-form tannate)
Merck Index: 623
Lednicer No.: 1 37, 70; 2 47

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 µL 100 µg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 µL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES

Column: 250 × 4.6 7 µm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM

Retention time: 8.0 (R-(-)), 9.5 (S-(+))

OTHER SUBSTANCES

Extracted: methamphetamine, tranylcypromine

KEY WORDS

plasma; derivatization; normal phase; chiral

REFERENCE

Weber,H.; Spahn,H.; Mutschler,E.; Möhrke,W. Activated α-alkyl-α-arylacetic acid enantiomers for stereoselective thin-layer chromatographic and high-performance liquid chromatographic determination of chiral amines, *J.Chromatogr.*, **1984**, *307*, 145–153.

SAMPLE

Matrix: blood
Sample preparation: 100 µL Serum + 50 µL 100 ng/mL aniline sulfate in water + 200 µL 20 mM pH 10.6 carbonate buffer + 2 mL ethyl acetate, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the organic layer and add it to 200 µL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the aqueous layer and add it to 40 µL 250 mM NaOH, add 50 µL 330 mM pH 7.8 phosphate buffer, add 250 µL MeCN, add 25 µL 1 mM (-)-1-(9-fluorenyl)ethyl chloroformate in acetone, let stand overnight at room temperature, add 30 µL 100 mM glycine in water, add 750 µL n-pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in MeCN:water 50:50, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: Direct-Connect column prefilter (Alltech)
Column: 150 × 4.6 3 µm Adsorbosphere HS C18 (Alltech)

Mobile phase: MeCN:THF:20 mM pH 3.6 acetate buffer 39:15:46
Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 22.6 (R), 23.6 (S)
Internal standard: aniline (21.0)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
serum; rat; chiral; derivatization

REFERENCE
Hutchaleelaha,A.; Walters,A.; Chow,H.-H.; Mayersohn,M. Sensitive enantiomer-specific high-performance liquid chromatographic analysis of methamphetamine and amphetamine from serum using precolumn fluorescent derivatization, *J.Chromatogr.B*, **1994**, *658*, 103–112.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM pH 11 borate buffer, mix, add 2.5 mL diethyl ether, vortex for 5 min, centrifuge at 1200 g for 5 min, remove organic layer, repeat extraction. Combine the organic layers and add them to 200 μL 100 mM HCl, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 150 μL 1 M pH 8 borate buffer and 100 μL 4 mM 9-fluorenylmethyl chloroformate in MeCN, shake, allow to react at 50° for 5 min, add 20 μL 20 mM proline in water, allow to react at 50° for 2 min, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeCN:50 mM pH 6.0 sodium phosphate buffer 50:50
Flow rate: 1
Injection volume: 200
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine, desmethyldeprenyl

KEY WORDS
plasma

REFERENCE
La Croix,R.; Pianezzola,E.; Strolin Benedetti,M. Sensitive high-performance liquid chromatographic method for the determination of the three main metabolites of selegiline (L-deprenyl) in human plasma, *J.Chromatogr.B*, **1994**, *656*, 251–258.

SAMPLE
Matrix: blood, tissue, dialysate
Sample preparation: Plasma. 45 μL Plasma + 5 μL 40 μM tryptamine + 100 μL 100 mM borate buffer adjusted to pH 10.6 with NaOH + 200 μL ethyl acetate, vortex for 2 min,

let sit on ice for 10 min, add 200 μL ethyl acetate, add 200 μL water, vortex briefly, centrifuge at 4° at 18000 g for 10 min. Remove 200 μL of the organic supernatant and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 80 μL 50 mM KH_2PO_4 adjusted to pH 2.6 with phosphoric acid, add 20 μL borate buffer adjusted to pH 11.5 with NaOH, add 20 μL reagent, let stand for at least 2 min, keep at 4°, inject a 75 μL aliquot. Tissue. Sonicate brain tissue with 9 volumes of 8 μM tryptamine in 100 mM pH 10.6 borate buffer for 20 s, centrifuge at 4° at 18000 g for 10 min, remove a 100 μL aliquot of the supernatant and add 200 μL ethyl acetate, vortex for 2 min, let sit on ice for 10 min, add 200 μL ethyl acetate, add 200 μL water, vortex briefly, centrifuge at 4° at 18000 g for 10 min. Remove 200 μL of the organic supernatant and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 80 μL 50 mM KH_2PO_4 adjusted to pH 2.6 with phosphoric acid, add 20 μL borate buffer adjusted to pH 11.5 with NaOH, add 20 μL reagent, let stand for at least 2 min, keep at 4°, inject a 75 μL aliquot. Dialysate. 100 μL Dialysate + 20 μL reagent, let stand for at least 2 min, keep at 4°, inject a 75 μL aliquot. (Reagent was 27 mg o-phthaldialdehyde in 500 μL EtOH, add 5 mL 100 mM pH 9.6 borate buffer, add 40 μL 3-mercaptopropionic acid, refrigerate, use for up to 4 days.)

HPLC VARIABLES

Guard column: 20 × 2 37-50 μm Bondapak C18/Corasil
Column: 250 × 4.6 5 μm LC-18 (Supelco)
Mobile phase: Gradient. MeOH:buffer 35:65 for 3 min, to 65:35 over 1 min, maintain at 65:35 for 14 min, return to initial conditions over 1 min, re-equilibrate for 6 min. (Buffer was 50 mM KH_2PO_4 adjusted to pH 5.5 with KOH.)
Flow rate: 1.5
Injection volume: 75
Detector: F ex 340 em 440

CHROMATOGRAM

Retention time: 14.5
Internal standard: tryptamine (13)
Limit of detection: 200 pg (plasma, tissue), 50 pg (dialysate)
Limit of quantitation: 100 pg (dialysate)

OTHER SUBSTANCES

Extracted: metabolites, p-hydroxyamphetamine

KEY WORDS

rat; brain; plasma; derivatization

REFERENCE

Bowyer,J.F.; Clausing,P.; Newport,G.D. Determination of d-amphetamine in biological samples using high-performance liquid chromatography after precolumn derivatization with o-phthaldialdehyde and 3-mercaptopropionic acid, *J.Chromatogr.B*, **1995**, *666*, 241–250.

SAMPLE

Matrix: blood, urine
Sample preparation: Adjust pH of 10 mL plasma or 20 mL urine to 11.4 with 5 M NaOH, add to a column containing 1.5 g Amberlite XAD-2, wash with 10 mL water, elute with 20 (plasma) or 40 (urine) mL chloroform:isopropanol 75:25, add 100 μL 6 M HCl in EtOH to the eluate, evaporate to dryness under reduced pressure, reconstitute with 1 mL 8% sodium bicarbonate, add 1 mL 0.5% sodium 1,2-naphthoquinone-4-sulfonate, heat at 70° for 20 min, add an equal volume of chloroform, vortex for 1 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES

Column: 150 × 5 Partisil 5
Mobile phase: Hexane:chloroform:ethyl acetate:EtOH 50:25:35:1

Column temperature: 20
Flow rate: 2.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 5
Internal standard: phenylethylamine (6)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: hydroxyamphetamine, methamphetamine, norephedrine

KEY WORDS
derivatization; plasma; normal phase; comparison with other derivatization reagents and with ion-pair chromatography; SPE

REFERENCE
Farrell,B.M.; Jefferies,T.M. An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines, *J.Chromatogr.*, **1983**, *272*, 111–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Adjust pH of 10 mL plasma or 20 mL urine to 11.4 with 5 M NaOH, add to a column containing 1.5 g Amberlite XAD-2, wash with 10 mL water, elute with 20 (plasma) or 40 (urine) mL chloroform:isopropanol 75:25, add 100 μL 6 M HCl in EtOH to the eluate, evaporate to dryness under reduced pressure, reconstitute with 1 mL EtOH, add 1 mL reagent, mix, filter, inject a 50 μL aliquot. (Prepare reagent by dissolving 200 mg o-phthalaldehyde in 2 mL MeOH, add 400 μL mercaptoethanol, add to buffer, store in the dark in the refrigerator, discard after 5 days. Prepare buffer by dissolving 1 g boric acid in 38 mL water and adjusting pH to 10.4 with 4 M KOH.)

HPLC VARIABLES
Column: 200 × 5 10 μm Partisil ODS-2
Mobile phase: MeOH:water 73:27 containing 0.2% EDTA
Column temperature: 20
Flow rate: 1.8
Injection volume: 50
Detector: F ex 345 em 445

CHROMATOGRAM
Retention time: 9
Internal standard: benzylamine (6)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: hydroxyamphetamine, norephedrine

KEY WORDS
derivatization; plasma; comparison with other derivatization reagents and with ion-pair chromatography; SPE

REFERENCE
Farrell,B.M.; Jefferies,T.M. An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines, *J.Chromatogr.*, **1983**, *272*, 111–128.

SAMPLE
Matrix: blood, urine

Sample preparation: Serum. 1 mL Serum + 1 mL 120 mM sodium dodecyl sulfate in 100 mM NaOH, homogenize, filter (45 μm). Inject on to column A at 180 μL/min 200 μL 60 mM sodium dodecyl sulfate in MeCN:water 50:50, 25 μL filtrate, and 25 μL 60 mM sodium dodecyl sulfate in MeCN:water 10:90, wait for 1 min, inject 100 μL 60 mM sodium dodecyl sulfate in MeCN:water 10:90, inject 200 μL 10 mM sodium dodecyl sulfate in MeCN:water 10:90, backflush the contents of column A on to column B with mobile phase, after 18 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with 300 μL 60 mM sodium dodecyl sulfate in MeCN:water 50:50 and 300 μL 10 mM sodium dodecyl sulfate in MeCN:water 80:20. Urine. 5 mL Urine + 4.5 mL MeCN + 500 μL 1 M KOH, centrifuge at 2500 rpm for 10 min, filter (45 μm) the supernatant. Inject on to column A at 180 μL/min 25 μL 50 mM KOH in MeCN:water 20:80, 50 μL filtrate, 25 μL 50 mM KOH in MeCN:water 20:80, and 200 μL MeCN:water 20:80, backflush the contents of column A on to column B with mobile phase, after 18 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with 400 μL MeCN.

HPLC VARIABLES

Column: A 20 × 2 polymeric reagent; B 250 × 4.6 5 μm Supelcosil LC-18-DB (with a guard column) (Prepare polymeric reagent as follows. Prepare a porous rigid resin using a divinylbenzene:ethylstyrene:styrene 24:6:70 mixture with trimethylsilyl modified silica (102 Å average pore size, 1.08 mL/g pore volume, 366 m^2/g surface area, 16-20 μm irregular particle shape, IMPAQ RG 1020 Si silica, PQ Co., Valley Forge PA). Further preparation details are not given but a typical procedure given in the cited reference is as follows. Aerate a mixture of 10 g modified silica in 100 mL water with nitrogen for 15 min, add 10 mL styrene:80% divinylbenzene:t-butyl peroxybenzoate 49:49:2 (remove preservative by passing through a butylcatechol remover (Scientific Polymer, Ontario NY), shake vigorously at room temperature for 4 h, add 150 mL 0.75% polyvinyl alcohol, shake for 4 h, heat at 120° for 24 h while shaking on a Parr instrument, cool to room temperature, filter, wash with 100 mL water, wash with 50 mL MeOH. Add the solid to 500 mL 3 M NaOH in MeOH:water 40:60, shake at room temperature for 14 h (to dissolve the silica), filter, wash with water until the washings are neutral, wash with 100 mL MeOH, dry at 60°. The polymer has similar properties to the template silica (US Pat. 4 933 372 (1990)). Soxhlet extract the resin with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

Mobile phase: Gradient. MeCN:water 50:50 for 3.5 min, to 70:30 over 12 min, maintain at 70:30 for 2.5 min, return to initial conditions over 1 min. (Place a 100 × 4.6 30-40 μm silica column before the injector.)

Column temperature: 60 (column A only)

Injection volume: 25-50

Detector: F ex 254 em 305-395

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 25 ng/mL (urine), 600 ng/mL (serum)

OTHER SUBSTANCES
Extracted: methamphetamine (only in urine)

KEY WORDS
derivatization; serum

REFERENCE
Bourque,A.J.; Krull,I.S.; Feibush,B. Automated HPLC analyses of drugs of abuse via direct injection of biological fluids followed by simultaneous solid-phase extraction and derivatization with fluorescence detection, *Biomed.Chromatogr.*, **1994**, *8*, 53–62.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.71

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Simultaneous: ephedrine, methamphetamine, phenmetrazine, phentermine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: bulk
Sample preparation: Mix 200 μmole amine with 500 μmole (1S)-(+)-camphor-10-sulfonyl chloride, 10 mL diethyl ether, and 10 mL 1 M NaOH, stir vigorously for 1 h, acidify with concentrated HCl, extract three times with diethyl ether. Combine the organic layers and wash them three times with water, evaporate to dryness, reconstitute with 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Silica 100-RP 18
Mobile phase: MeOH:water 50:50
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 15.55, 18.13 (enantiomers)

OTHER SUBSTANCES
Also analyzed: bamethan, ephedrine, norpseudoephedrine, 1-phenylethylamine

KEY WORDS
derivatization; chiral

REFERENCE
Vogt,C.; Jira,T.; Beyrich,T. HPLC-Trennung racemischer Amine nach Derivatisierung mit (1S)-(+)-Campher-10-sulfonylchlorid, *Pharmazie*, **1990**, *45*, 691.

SAMPLE
Matrix: bulk
Sample preparation: Reflux a 10% excess of reagent in toluene for 10 min, add the drug, let stand at room temperature for 10 min, cool, dilute, inject an aliquot. (The reagent was N-(p-toluenesulfonyl)prolyl azide and was prepared as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles p-toluenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above

for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(p-toluene)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!) (Anal.Chem. 1984, 56, 958). Suspend 86 mmoles 1-[(p-toluene)sulfonyl]proline in 15 mL water and add sufficient acetone to give a clear solution, cool to 0°, add 10.2 g triethylamine in 175 mL acetone, slowly add 12.5 g ethyl chloroformate in 45 mL acetone while maintaining the temperature at 0°, stir at 0° for 30 min, add dropwise 8.6 g sodium azide in 30 mL water, stir at 0° for 1 h, pour into ice water, extract with ether, dry over anhydrous magnesium sulfate, evaporate under reduced pressure at room temperature to give N-(p-toluenesulfonyl)prolyl azide (cf J.Org.Chem. 1961, 26, 3511).)

HPLC VARIABLES
Column: 300 × 4 7-9 μm silica gel
Mobile phase: Petroleum ether:isopropanol 96.5:3.5
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 13, 16 (enantiomers)

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Zhou,Y.; Sun,Z.P.; Lin,D.K. Liquid chromatographic evaluation of a new chiral derivatizing agent for enantiomeric resolution of amine and alcohol drugs, *J.Liq.Chromatogr.*, **1990**, *13*, 875–885.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 45:55
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 11.86, k' 12.48 (enantiomers)

OTHER SUBSTANCES
Simultaneous: normetoprolol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: bulk, formulations
Sample preparation: 10 mg Bulk drug, tablet, or capsule or 10 mL 1 mg/mL syrup + 5 mL 20% NaOH, sonicate until dissolved, add 10 mL 10 mM 2 naphthoyl chloride in dichloromethane, shake for 1 min. Remove the organic phase and wash the aqueous phase with 5 mL dichloromethane. Combine the organic layers and wash them with two 5 mL portions of 10 mM sulfuric acid, filter through a cotton plug, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 35-50 μm silica
Column: 250 × 4.6 Pirkle Covalent Phenylglycine (Regis)
Mobile phase: Hexane:isopropanol:MeCN 97:3:0.5
Column temperature: 20
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: k' 20 (-), k' 22 (+)

KEY WORDS
tablets; capsules; syrup; chiral; derivatization; 1% of minor enantiomer can be detected

REFERENCE
Wainer,I.W.; Doyle,T.D.; Adams,W.M. Liquid chromatographic chiral stationary phases in pharmaceutical analysis: determination of trace amounts of the (-)-enantiomer in (+)-amphetamine, *J.Pharm.Sci.*, **1984**, *73*, 1162–1164.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Dissolve 10 mg bulk drug in 5 mL dichloromethane, add 5 mL 20% NaOH, add 10 mL 10 mM 2-naphthoyl chloride in dichloromethane, shake for 1 min. Remove the organic phase and wash the aqueous phase with 5 mL dichloromethane. Combine the organic layers and wash them with 5 mL 10 mM sulfuric acid. Filter the organic layer through a syringe containing a glass wool plug and anhydrous sodium sulfate, inject a 10 μL aliquot. Capsules. Sonicate 1 capsule in 5 mL 20% NaOH for 45 min or until dissolved, filter, add 5 mL dichloromethane to filtrate, add 10 mL 10 mM 2-naphthoyl chloride in dichloromethane, shake for 1 min. Remove the organic phase and wash the aqueous phase with 5 mL dichloromethane. Combine the organic layers and wash them with 5 mL 10 mM sulfuric acid. Filter the organic layer through a syringe containing a glass wool plug and anhydrous sodium sulfate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: Bakerbond Chiral Phase [DNBPG]
Mobile phase: Hexane:isopropanol:MeCN 97:3:0.5
Column temperature: 20

Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 28.5 (l), 31 (d)

KEY WORDS
capsules; chiral; derivatization

REFERENCE
Alembik,M.C.; Wainer,I.W. Resolution and analysis of enantiomers of amphetamines by liquid chromatography on a chiral stationary phase: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 530–533.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM
Retention time: 3.62
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: ephedrine, methamphetamine, caffeine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: meconium
Sample preparation: 500 mg Meconium + 5 mL water + 1 drop 500 mM HCl, vortex for 1 min, sonicate for 5 min, vortex for 1 min, centrifuge at 2683 g for 10 min. Remove the supernatant and add it to 5 mL water and 1 drop 500 mM HCl, vortex for 1 min, sonicate for 5 min, vortex for 1 min, centrifuge at 2683 g for 10 min. Make up the supernatant to 20 mL with pH 9.0 borax buffer, add it to an Extrelut SPE cartridge, after 10 min elute with 60 mL dichloromethane:isopropanol 80:20. Add 1 drop 2% tartaric acid in water and evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18 DB

Mobile phase: MeOH:MeCN:THF:triethylamine:water 100:25:7:1.5:600 containing 77 mM KH_2PO_4
Flow rate: 1
Injection volume: 20
Detector: UV 204

CHROMATOGRAM
Retention time: k' 4.3
Limit of detection: 500 ng/g

OTHER SUBSTANCES
Extracted: morphine
Noninterfering: caffeine, benzoylecgonine, cocaine, codeine

KEY WORDS
SPE

REFERENCE
Franssen,R.M.E.; Stolk,L.M.L.; van den Brand,W.; Smit,B.J. Analysis of morphine and amphetamine in meconium with immunoassay and HPLC-diode-array detection, *J.Anal.Toxicol.*, **1994**, *18*, 294–295.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL 20-300 μg/mL amine solution in water with 2 mL 50 mg/mL 4-nitrobenzoyl chloride in THF (freshly prepared) and 1 mL 1 M NaOH, heat at 65° for 1 h, cool, adjust pH to 12 with 1 M NaOH, extract with two 10 mL portions of chloroform. Combine the extracts and wash them with two 20 mL portions of 10% potassium carbonate, wash with water, dry over anhydrous magnesium sulfate. Evaporate to dryness under a stream of air, reconstitute the residue in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 35:65
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: benzylamine, methamphetamine, α-methylbenzylamine, n-propylamphetamine

KEY WORDS
derivatization

REFERENCE
Clark,R.C.; Teague,J.D.; Wells,M.M.; Ellis,J.H. Gas and high-pressure liquid chromatographic properties of some 4-nitrobenzamides of amphetamines and related arylalkylamines, *Anal.Chem.*, **1977**, *49*, 912–915.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.62

OTHER SUBSTANCES

Simultaneous: morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL 1 mg/mL Compound in dichloromethane + 50 μL 7.6 mM 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in MeCN or dichloromethane: triethylamine 99.8:0.2, mix, let stand at room temperature for 1 h, add 1 mL 1 M HCl, shake mechanically for 5 min, centrifuge at 1000 g for 15 min, discard the aqueous phase, add 1 mL 1 M NaOH, shake for 5 min, centrifuge at 1000 g for 15 min. Remove a 10 μL aliquot of the organic layer and dilute it to 1 mL with MeOH, inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm octadecyl (IBM)
Mobile phase: MeOH:water 55:45
Flow rate: 1-2
Injection volume: 20-50
Detector: UV 254

CHROMATOGRAM

Retention time: 16.8 (S), 18.2 (R)
Limit of quantitation: 100 ng

OTHER SUBSTANCES

Simultaneous: 1-(4-chlorophenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, 1-(2,4-dimethoxy-5-methylphenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-thiomethylphenyl)-2-aminopropane, 1-phenylethylamine

KEY WORDS
derivatization; comparison with other derivatizing reagents; chiral

REFERENCE
Miller,K.J.; Gal,J.; Ames,M.M. High-performance liquid chromatographic resolution of enantiomers of 1-phenyl-2-aminopropanes (amphetamines) with four chiral reagents, *J.Chromatogr.*, **1984**, *307*, 335–342.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL 1 mg/mL Compound in dichloromethane + 100 μL 6.8 mM (R)-(+)-1-phenylethylisocyanate in dichloromethane, mix, let stand at room temperature for 1 h, evaporate to dryness under a stream of nitrogen, add 1 mL 100 mM NaOH, vortex for 15 min, add 1 mL 20% NaOH, add 3 mL dichloromethane, shake mechanically for 15 min, centrifuge at 1000 g for 15 min. Remove the organic layer and wash it with 2 mL 100 mM HCl. Remove a 100 μL aliquot of the organic layer and dilute it to 1 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm octadecyl (IBM)
Mobile phase: MeOH:water 60:40
Flow rate: 1-2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13.5 (R), 14.0 (S)
Limit of quantitation: 100 ng

OTHER SUBSTANCES
Simultaneous: 1-(4-chlorophenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, 1-(2,4-dimethoxy-5-methylphenyl)-2-aminopropane, 1-phenylethylamine

KEY WORDS
derivatization; comparison with other derivatizing reagents; chiral

REFERENCE
Miller,K.J.; Gal,J.; Ames,M.M. High-performance liquid chromatographic resolution of enantiomers of 1-phenyl-2-aminopropanes (amphetamines) with four chiral reagents, *J.Chromatogr.*, **1984**, *307*, 335–342.

SAMPLE
Matrix: solutions
Sample preparation: 2 mL THF + 1 mL 33.5 mM reagent in THF (freshly prepared) + 1 mL 1 mg/mL amphetamine in water + 700 μL 10% sodium bicarbonate in water, heat at 65° for 1 h, cool, extract three times with 10 mL aliquots of chloroform. Combine the extracts and wash them with 10 mL water, dry over anhydrous magnesium sulfate, evaporate to dryness, reconstitute with 2.5 mL mobile phase, inject a 5 μL aliquot. (Prepare reagent (1-[(4-nitrophenyl)sulfonyl]prolyl chloride) as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrobenzenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(4-nitrophenyl)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles 1-[(4-nitrophenyl)sulfonyl]proline in 100 mL benzene and add 75 mmoles thionyl chloride in 50 mL benzene dropwise, heat at 35-40° until the

reaction is complete (about 48 h; monitor by IR), evaporate to dryness, recrystallize from n-heptane to give 1-[(4-nitrophenyl)sulfonyl]prolyl chloride (Anal.Chem. 1984, 56, 958) (mp 110-110.5°).)

HPLC VARIABLES
Guard column: 70 × 2.1 30-38 μm HC Pellosil (Whatman)
Column: 150 × 4.6 5 μm Supelcosil LC-Si
Mobile phase: n-Heptane:chloroform 20:80
Flow rate: 1.4
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4 (R), 4.5 (S)

KEY WORDS
derivatization; normal phase; chiral

REFERENCE
Barksdale,J.M.; Clark,C.R. Liquid chromatographic determination of the enantiomeric composition of amphetamine and related drugs by diastereomeric derivatization, *J.Chromatogr.Sci.*, **1985**, *23*, 176–180.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine, ephedrine, hydroxyamphetamine, methamphetamine, phentermine, mephentermine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 2 mg in 15 mL 0.45 M NaOH, extract twice with 30 mL chloroform. Combine the organic layers and add a 10% molar excess of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in chloroform, let stand for 10 min, evaporate the chloroform under a stream of air, dissolve the residue in 1 mL THF or MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water:acetic acid 50:49:1
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 25 (R), 28 (S)

OTHER SUBSTANCES
Simultaneous: norpseudoephedrine, norephedrine

KEY WORDS
chiral; derivatization

REFERENCE
Noggle,F.T.,Jr.; DeRuiter,J.; Clark,C.R. Liquid chromatographic determination of the enantiomeric composition of amphetamine prepared from norephedrine and norpseudoephedrine, *J.Chromatogr.Sci.*, **1987**, *25*, 38–42.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Simultaneous: acetophenone, desipramine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions

Sample preparation: Mix sample:50 mM (?) NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 5:95. B was MeCN:MeOH:50 mM pH 6.8 potassium phosphate buffer 55:10:35. A:B from 70:30 to 0:100 over 1 h, maintain at 0:100 for 20 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 65

OTHER SUBSTANCES
Simultaneous: baclofen, tranylcypromine

KEY WORDS
derivatization

REFERENCE
Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE
Matrix: solutions
Sample preparation: Mix 500 μL of a solution in 20 mM pH 9.5 borate buffer with 100 μL 10 mM NaCN in 20 mM pH 9.5 borate buffer, add 500 μL 0.1 mM naphthalene-2,3-dicarboxaldehyde in MeOH, mix, let stand at room temperature for 20 min, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.1 5 μm LiChrosorb RP-18
Mobile phase: MeCN:2.5 mM pH 7.0 imidazole buffer 75:25
Flow rate: 0.5
Injection volume: 25
Detector: Chemiluminescence (418 nm cutoff filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN containing 5 mM bis(2-nitrophenyl)oxalate pumped at 0.2 mL/min and the mixture flowed into the detector.

CHROMATOGRAM
Limit of detection: 4 fmole

KEY WORDS
derivatization

REFERENCE
Kwakman,P.J.M.; Koelewijn,H.; Kool,I.; Brinkman,U.A.T.; de Jong,G.J. Naphthalene- and anthracene-2,3-dialdehyde as precolumn labelling reagents for primary amines using reversed- and normal-phase liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1990**, *511*, 155–166.

SAMPLE
Matrix: solutions

Sample preparation: Inject a 10 μL aliquot of a 100 ppm solution in MeCN:dichloromethane:triethylamine 50:50:0.05 into the mobile phase. The mobile phase flows through a 27 × 2.2 reactor packed with reagent at 72° to the column. (Reagent was dinitrobenzoyl-o-nitrobenzophenone polymeric reagent, prepared as follows. (Caution! Dioxane is carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) Soxhlet extract 200-400 mesh polystyrene cross-linked with 4% divinylbenzene (Fluka) with MeCN for 48 h. Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to a mixture of 50 g of the polystyrene resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir the mixture mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice. Wash the beads with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm DMF (60°), wash with six 300 mL portions of dichloromethane:MeOH 2:1, dry. Add the polymer to 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane, heat at 90° for 8 h, filter, repeat the process, filter, wash the beads with four portions of warm (60°) dioxane, add 30 mL acetic acid, stir for 15 min, wash with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-(R)-Naphthyl Urea (Supelco)
Mobile phase: Hexane:dichloromethane:THF 70:27:3
Flow rate: 0.1 for 40 s, to 3.1 over 30 s, maintain at 3.1
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: 11.5 (D), 13 (L)

KEY WORDS

derivatization; chiral

REFERENCE

Bourque,A.J.; Krull,I.S. Solid-phase reagent containing the 3,5-dinitrophenyl tag for the improved derivatization of chiral and achiral amines, amino alcohols and amino acids in high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1991**, *537*, 123–152.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 2.220

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 200 ppm solution in MeCN:500 mM pH 9.0 borate buffer 50:50 with 25 mg reagent, after 1 min elute with 1 mL hexane:THF 75:25, inject a 5 μL aliquot. (Reagent was dinitrophenyl carbamate benzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Dissolve 3,5-dinitrobenzoyl chloride in the minimum amount of glacial acetic acid, add an equimolar amount of sodium azide, stir for 30 min, dilute with water, filter to obtain 3,5-dinitrobenzoyl azide (Caution! Azides are toxic and potentially explosive!) (J. Liq. Chromatogr. 1986, 9, 443). Heat 71 mg 3,5-dinitrobenzoyl azide in 15 mL toluene (dried over calcium hydride) at ??? for 30 min, cool using an ice bath, add 200 mg polymer, allow to warm to room temperature with stirring for 1 h, filter, wash the polymer with four 10 mL portions of warm (40°) dichloromethane, dry under high vacuum for 1 h.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-(R)-naphthylurea (Supelco)
Mobile phase: Hexane:EtOH:MeCN 93:7:0.5
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 12.2, 15.7 (enantiomers)

OTHER SUBSTANCES

Simultaneous: methamphetamine

KEY WORDS

derivatization; chiral

REFERENCE

Bourque,A.J.; Krull,I.S. Immobilized isocyanates for derivatization of amines for chiral recognition in liquid chromatography with UV detection, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 495–503.

SAMPLE

Matrix: solutions

Sample preparation: 50 μL 5 mg/mL Amphetamine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 21.8 mg 1-thio-β-D-glucose in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:MeCN:buffer 55:2:45 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 425 or UV 254

CHROMATOGRAM
Retention time: 12.37 (R-(-)), 14.12 (S-(+))

KEY WORDS
derivatization; protect from light; chiral

REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: diethylpropion, phenylpropanolamine, ephedrine, methamphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL aliquot of a 0.1-1 mM solution in 10 mM HCl with 500 μL 100 mM pH 9.5 borate buffer, 100 μL 2.5 mM N-acetyl-L-cysteine in 10 mM HCl, and 200 μL 5 mM o-phthalaldehyde in EtOH, let stand for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18 end-capped
Mobile phase: MeOH:50 mM pH 6.5 phosphate buffer 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 335

CHROMATOGRAM
Retention time: k' 3.5 (α = 1.13, R_s = 1.04)

KEY WORDS
derivatization; chiral; comparison with capillary electrophoresis; comparison with other derivatizing reagents

REFERENCE
Leroy,P.; Bellucci,L.; Nicolas,A. Chiral derivatization for separation of racemic amino and thiol drugs by liquid chromatography and capillary electrophoresis, *Chirality*, **1995**, 7, 235–242.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL Solution + 500 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water + 500 μL buffer, let stand for 10 min, extract three times with 2 mL aliquots of n-hexane:ethyl acetate 50:50. Combine the organic layers and evaporate them to dryness at 80°, reconstitute with 2 mL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS C18
Mobile phase: Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: solutions, urine
Sample preparation: Add 20 μL of a solution of amphetamine in MeCN:water 50:50 to a cartridge containing 30 mg reagent, heat at 60° for 5 min, elute with 500 μL MeCN, inject a 20 μL aliquot of the eluate. Alternatively, inject 10 μL of urine (adjusted to pH 10 with 100 mM NaOH and filtered) into a 27 × 3 reactor filled with reagent held at 60°, after 5 min elute the contents of the reactor on to the column with mobile phase, elute the column with mobile phase, monitor the effluent from the column. (Prepare reagent as follows. Add 3 g aluminum trichloride to 5 g 60-90 μm macroporous polystyrene-divinylbenzene

copolymer (Fluka) and 10 g 4-chloro-3-nitrobenzoyl chloride stirred in 100 mL dry nitrobenzene, stir at 60° for 5 h, pour into a mixture of 75 mL DMF, 50 mL concentrated HCl, and 50 g ice, filter. Wash the solid with three 50 mL portions of DMF:water 75:25, wash with 30 mL warm (60°) DMF, wash with five 50 mL portions of dichloromethane:MeOH 2:1. Stir the product in 15 mL 40% benzyltrimethylammonium hydroxide in water, 15 mL water, and 30 mL dioxane (Caution! Dioxane is a carcinogen!) at 90° for 8 h, filter. Wash the product with four 50 mL portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with three 50 mL portions of dioxane until the washings are neutral, wash with four 50 mL portions of dichloromethane:MeOH 2:1. Stir 1 g of the yellow product in 10 mL dichloromethane and 500 μL pyridine, add 1.24 g 9-fluorenylmethyl chloroformate, stir at room temperature for 30 min, filter. Wash the solid with three 20 mL portions of DMF, three 20 mL portions of dichloromethane, and three 20 mL portions of dry diethyl ether, dry under vacuum to give the reagent (polymer bound o-nitrobenzophenone fluorenylmethyl carbonate) Store in the freezer, good for at least a year.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChroSpher C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.5
Injection volume: 20
Detector: F ex 265 em 320

CHROMATOGRAM

Retention time: 5
Limit of detection: 100 ppb

OTHER SUBSTANCES

Also analyzed: butylamine, diethylamine, morpholine, propylamine

KEY WORDS

derivatization

REFERENCE

Gao,C.-X.; Chou,T.-Y.; Krull,I.S. Polymeric activated ester reagents for off-line and on-line derivatizations of amine nucleophiles in high-performance liquid chromatography with ultraviolet and fluorescence detection, *Anal.Chem.*, **1989**, *61*, 1538–1548.

SAMPLE

Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)
Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.
Flow rate: 1
Injection volume: 50
Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 136

CHROMATOGRAM

Retention time: 14.7

Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES

Extracted: 6-acetylmorphine, benzoylecgonine, cocaine, ephedrine, methamphetamine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS

SPE

REFERENCE

Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 2.5

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam

Interfering: phenylpropanolamine, phentermine, phenmetrazine, lidocaine, ephedrine, pentazocine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of urine to 10 with 1 M NaOH, inject a 10 μL aliquot onto column A heated to 60°, after 6 s stop the flow through column A, after 5 min backflush the contents of column A onto column B with mobile phase, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 27 × 30 packed with polymeric reagent; B 250 × 4 5 μm LiChrospher C18 (Prepare polymeric reagent, polymeric FMOC-L-proline, as follows. Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g 60-90 μm 96% styrene-4% divinylbenzene resin (Fluka) and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Dissolve 600 mg N-(9-fluorenylmethoxycarbonyl)-L-proline in 10 mL dichloromethane, cool to 0°, add 1.8 mmoles dicyclohexylcarbodiimide, stir at 0° for 30 min, filter. Add the filtrate to 1 g resin, add 500 μL pyridine, stir at room temperature for 1 h, filter, wash with three 50 mL portions of hexane, wash with three 50 mL portions of dichloromethane, wash with three 100 mL portions of MeCN.)
Mobile phase: MeCN:water 48:52
Flow rate: 1.5
Injection volume: 10
Detector: UV 265, F ex 265 em 315

CHROMATOGRAM
Retention time: 28 (d), 30 (l)
Limit of detection: 50 ng/mL

KEY WORDS
derivatization; chiral; column-switching

REFERENCE
Gao,C.-X.; Krull,I.S. Determination of enantiomeric drugs in physiological fluids using on-line solid phase derivatizations and reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1183–1198.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 100 μL 25 μg/mL N-n-propylaniline + 6 mL pH 10.0 carbonate buffer + 15 mL water, add mixture to an Extrelut SPE cartridge, let stand for 20 min, elute with 40 mL hexane:ethyl acetate 90:10. Add the eluate to 3 mL 100 mM sulfuric acid and 500 mg NaCl, stir for 20 min, centrifuge at 1000 g for 5 min. Remove the lower layer and add it to 3 mL 2.5 M NaOH and 20 μL benzoyl chloride, stir vigorously

for 30 min. Extract the mixture with 1.5 mL chloroform. Wash the chloroform layer twice with 5 mL water and evaporate it to dryness at 40°, reconstitute the residue in 200 μL hexane:isopropanol 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: Hexane:isopropanol 90:10
Column temperature: 48
Flow rate: 1-1.4
Detector: UV 220

CHROMATOGRAM
Retention time: 17 (l), 25 (d)
Internal standard: N-n-propylaniline (11)
Limit of detection: 25 ng

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
rat; SPE; derivatization; chiral

REFERENCE
Nagai,T.; Kamiyama,S. Assay of the optical isomers of methamphetamine and amphetamine in rat urine using high-performance liquid chromatography with chiral cellulose-based columns, *J.Chromatogr.*, **1990**, *525*, 203–209.

SAMPLE
Matrix: urine
Sample preparation: Mix 100 μL urine with 60 mg reagent, after 2 min elute with 500 μL MeCN, add 500 μL 50 mM NaOH to the eluate, mix, inject a 20 μL aliquot. (The reagent was dinitrobenzoylbenzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl: dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb C18
Mobile phase: MeCN:water 50:50 containing 0.05% ammonium hydroxide
Flow rate: 2
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: 4.1

KEY WORDS
derivatization

REFERENCE
Bourque,A.J.; Krull,I.S. Solid-phase reagent containing the 3,5-dinitrophenyl tag for the improved derivatization of chiral and achiral amines, amino alcohols and amino acids in high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1991**, *537*, 123–152.

SAMPLE
Matrix: urine
Sample preparation: Inject a 10 μL aliquot into a 27 × 2 reactor packed with 65 mg polymeric reagents A and B in a 5:1 ratio heated at 60°, after 10 min flush the contents of the reactor onto the column with the mobile phase. (Prepare the polymeric reagents as follows. Stir mechanically 5 g 60-90 μm polystyrene-divinylbenzene copolymer (Fluka), 10 g 4-chloro-3-nitrobenzoyl chloride, and 3 g aluminum trichloride in 100 mL dry nitrobenzene at 60° for 5 h, pour into a mixture of 75 mL DMF, 50 mL concentrated HCl, and 50 g ice, filter. Wash the solid with three 50 mL portions of DMF:water 75:25, wash with warm (60°) DMF, wash with three 50 mL portions of dichloromethane:MeOH 2:1. Add the solid to 15 mL 40% benzyltrimethylammonium hydroxide in water, 15 mL water, and 30 mL dioxane (Caution! Dioxane is a carcinogen!), heat at 90° for 8 h, filter, wash with four 50 mL portions of warm (60°) dioxane. Add 30 mL acetic acid:water 50:50 and stir for 15 min, filter, wash with water until the washings are neutral, wash the three 50 mL portions of dioxane, wash with four 50 mL portions of dichloromethane:MeOH 2:1. Stir 1.12 g of this product with 248 mg 9-fluorenylmethyl chloroformate in 2 mL dichloromethane and 100 μL pyridine at room temperature for 30 min, filter, wash with three 4 mL portions of DMF, wash with three 4 mL portions of dichloromethane, wash with three 4 mL portions of dry ethyl ether, dry under vacuum to give polymer-bound fluorenylmethoxycarbonyl reagent (A). Substitute 4-nitrobenzoyl chloride for 9-fluorenylmethyl chloroformate to obtain polymer-bound 4-nitrobenzoyl reagent (B).)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher C18
Mobile phase: MeCN:water 55:45
Flow rate: 1.5 for 6 min then 2.5
Injection volume: 10
Detector: UV 265, F ex 265 em 320

CHROMATOGRAM
Retention time: 6 (4-nitrobenzoyl derivative), 15 (fluorenylmethoxycarbonyl derivative)
Limit of quantitation: 10 ppm (F)

KEY WORDS
derivatization

REFERENCE
Gao,C.X.; Schmalzing,D.; Krull,I.S. A mixed-bed, multi-derivatization approach using polymeric reagents for derivatizations of amines in high performance liquid chromatographic detection, *Biomed.Chromatogr.*, **1991**, *5*, 23–31.

SAMPLE
Matrix: urine
Sample preparation: 200-500 μL Rat urine + 200-500 μL pH 3.8 acetate buffer + 25 μL 40 μg/mL β-glucuronidase and 20 μg/mL arylsulfatase (Merck), heat at 37° for 24 h, add 100 μL 25 μg/mL 3-methoxytyramine in water, add 100 μL water, adjust pH to 9.0 with 1.9 M sodium carbonate, add to an Extrelut SPE cartridge, let stand for 20 min, elute with 6 mL ethyl acetate. Add the eluate to 1 mL 100 mM sulfuric acid, extract. Add the aqueous layer to 3 mL 2.5 M NaOH, add 25 μL benzoyl chloride, extract with 5 mL ethyl

acetate. Wash the ethyl acetate layer with water, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL EtOH, 3.5 mL 50 mM pH 8.0 Tris/HCl buffer, and 35 μL esterase (Type 1 porcine liver, Sigma). Heat at 25° for 45 min, add to an activated Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 5 mL acetone. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL hexane:EtOH 89:11, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: n-Hexane:EtOH 89:11
Column temperature: 48
Flow rate: 1.4
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 12 (L), 14 (D)
Internal standard: 3-methoxytyramine (54)

OTHER SUBSTANCES
Extracted: metabolites, methamphetamine

KEY WORDS
rat; SPE; derivatization

REFERENCE
Nagai,T.; Kamiyama,S. Simultaneous HPLC analysis of optical isomers of methamphetamine and its metabolites, and stereoselective metabolism of racemic methamphetamine in rat urine, *J.Anal.Toxicol.*, **1991**, *15*, 299–304.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 3 mL urine to 11 with 10 M KOH, add to an Extrelut 3 column, let stand for 10 min, elute with 15 mL n-hexane into a tube containing one drop of 3 M HCl. Evaporate the eluate to dryness under a stream of nitrogen at 35°. Add 1.5 mL 8% sodium bicarbonate in water and 1 mL 0.5% sodium naphthoquinone-4-sulfonate in water to the residue, heat at 70° for 20 min, cool, extract with 5 mL carbon tetrachloride. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: MeCN:buffer 55:45 (Buffer was 1.361 g KH_2PO_4 in 950 mL, add 1.3 mL methanesulfonic acid, adjust pH to 3 with 5 M KOH, make up to 1 L with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 460

CHROMATOGRAM
Retention time: 7.3
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone,

naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS
SPE; derivatization

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Adsorbex SCX cation-exchange SPE cartridge (Merck) with 2 mL MeOH, 1 mL water, and 1 mL 17 mM KH_2PO_4, do not allow to dry. Centrifuge urine at 2000 g for 5 min. 1 mL Urine + 500 μL 50 mM KH_2PO_4, sonicate for 1 min, add to the SPE cartridge, rinse vial with 50 μL 50 mM KH_2PO_4 and add to cartridge, dry cartridge for 1 min, wash with three 500 μL portions of 17 mM KH_2PO_4, wash with 1 mL MeOH, dry under vacuum for 1 min, elute with four 500 μL portions of MeOH: 7.3% HCl (97.5:2.5) at a flow rate of 0.5 mL/min, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL (8.5 g) 85% orthophosphoric acid and 280 μL (0.22 g) hexylamine per liter. B was MeCN containing 100 mL water, 5 mL (8.5 g) 85% orthophosphoric acid, and 280 μL (0.22 g) hexylamine per liter. A:B 94.5:5.5 for 10.6 min, then to 61:39 over 11 min.
Column temperature: 40
Flow rate: 0.8
Injection volume: 10
Detector: UV 198

CHROMATOGRAM
Retention time: 6
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: 3,4-methylenedioxyamphetamine, methamphetamine, 4-methoxyamphetamine, phentermine, 3,4-methylenedioxymethamphetamine, 5-methoxy-3,4-methylenedioxyamphetamine, 3,4,5-trimethoxyamphetamine, 3,4-methylenedioxyethylamphetamine, 2,5-dimethoxyamphetamine, 4-bromo-2,5-dimethoxyphenylethylamine, 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine, 2,5-dimethoxy-4-ethylamphetamine, mescaline, methoxamine

KEY WORDS
SPE

REFERENCE
Helmlin,H.-J.; Brenneisen,R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1992**, *593*, 87–94.

SAMPLE
Matrix: urine
Sample preparation: Condition a 130 mg Bond Elut Certify SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 6.0 phosphate buffer, do not allow to go dry. 2 mL Urine +

800 μL 100 mM pH 6.0 phosphate buffer + 200 μL 10 μg/mL 1-methyl-3-phenylpropylamine in MeOH, if necessary adjust pH to 5-7 with 1 M KOH or 1 M HCl (with pH paper), add the mixture to the SPE cartridge, wash with 1 mL 1 M acetic acid, wash with 3 mL water, dry for 5 min under vacuum, wash with 6 mL MeOH, dry for 2 min under vacuum, elute with 2 mL ethyl acetate:30% ammonium hydroxide 98:2 at a flow rate of 4-6 drops/sec. Evaporate the eluate for 2 min under a stream of nitrogen, add 100 μL 1 M HCl in diethyl ether, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 125 μL MeCN:water:triethylamine 90:8.6:1.4. Add the entire volume to 50 ± 5 mg reagent in a 1 mL plastic pipette tip plugged with a Kimwipe, react for 30 s, elute with 500 μL MeCN under pressure, inject a 10-20 μL aliquot. (The reagent was dinitrobenzoylbenzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-Dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Add a portion of polymer to dry chloroform, add a three-fold excess of 3,5-dinitrobenzoyl chloride and pyridine, stir at 0-10° for 30 min, filter off polymer, wash with chloroform to give the reagent (J.Org.Chem. 1984, 49, 922).)

HPLC VARIABLES

Guard column: 20 mm long Microsorb octadecyldimethylsilyl silica (Rainin)
Column: 10 (sic)x 4.6 5 μm Microsorb octadecyldimethylsilyl silica (Rainin)
Mobile phase: MeCN:10 mM pH 2.5 phosphate buffer 45:55
Flow rate: 0.7
Injection volume: 10-20
Detector: UV 220

CHROMATOGRAM

Retention time: 16.3
Internal standard: 1-methyl-3-phenylpropylamine (23.0)
Limit of detection: 14 ng/mL
Limit of quantitation: 47 ng/mL

OTHER SUBSTANCES

Noninterfering: benzoylecgonine, cocaine, codeine, glutethimide, imipramine, meperidine, methadone, methamphetamine, methaqualone, morphine, nortriptyline, oxazepam, phencyclidine, propoxyphene, quinine

KEY WORDS

SPE; derivatization

REFERENCE

Fisher,D.H.; Bourque,A.J. Quantification of amphetamine in urine: solid-phase extraction, polymeric reagent derivatization and reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 142–147.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B
Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 75:25 over 7 min, hold at 75:25 for 3 min, return to 10:90 over 5 min, equilibrate at 10:90 for 5 min
Flow rate: 1.5
Injection volume: 20
Detector: UV 190-370

CHROMATOGRAM

Retention time: 4.9

OTHER SUBSTANCES

Extracted: amitriptyline, morphine, codeine, benzoylecgonine, meperidine, norpropoxyphene, nordiazepam
Also analyzed: phenylpropanolamine, lidocaine, diphenhydramine, nortriptyline, ephedrine, cocaine (different gradient).

REFERENCE

Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

SAMPLE

Matrix: urine
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 500 μL MeOH and 500 μL water. Adjust pH of urine to 10, centrifuge at 1500 g. 2 mL Supernatant + 100 μL 75 μg/mL β-phenylethylamine hydrochloride in water, add to the SPE cartridge, wash with 2.5 mL water, elute with 2 mL MeOH, evaporate the eluate to dryness. Reconstitute in water, add 500 μL 8% sodium bicarbonate, add 500 μL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, make up to 1.5 mL with water, heat at 70° for 20 min, cool, add an equal volume of chloroform, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and dry it over anhydrous sodium sulfate, filter (0.45 μm), inject a 25 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher Si-60
Mobile phase: EtOH:chloroform:ethyl acetate:n-hexane 1:22:32:45
Flow rate: 2
Injection volume: 25
Detector: UV 280

CHROMATOGRAM

Retention time: 3.7
Internal standard: β-phenylethylamine hydrochloride (4.9)

OTHER SUBSTANCES

Extracted: methamphetamine

KEY WORDS

SPE; normal phase; derivatization

REFERENCE

Campins Falcó,P.; Molins Legua,C.; Herráez Hernandez,R.; Sevillano Cabeza,A. Improved amphetamine and methamphetamine determination in urine by normal-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulphonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *663*, 235–245.

SAMPLE

Matrix: urine

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 50 mM pH 11 phosphate buffer. 500 μL Urine + 500 μL 2000 U/mL β-glucuronidase with sulfatase activity (Type H-1, Sigma) in 100 mM pH 5 acetate buffer, heat at 37° overnight, add 500 mg NaCl, add 500 μL 50 mM pH 11 potassium phosphate buffer, add 1.3 μg 4'-hydroxymethamphetamine, add 3.1 μg methamphetamine, adjust pH to 11 with ammonium hydroxide, mix. Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 50 mM pH 11 phosphate buffer. Add the mixture to the SPE cartridge, wash with 1 mL 50 mM pH 11 potassium phosphate buffer, wash with 1 mL freshly prepared MeOH:water 30:70, wash with 1 mL MeCN, elute with 1 mL freshly prepared MeCN: acetic acid 98:2, elute with 1 mL MeCN:HCl 98:2. Combine the eluates and evaporate them to dryness under a stream of air at room temperature, reconstitute the residue in mobile phase (?), inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: phenyl
Column: Microsorb phenyl
Mobile phase: MeCN:MeOH:50 mM pH 3 potassium phosphate 5:10:85
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM

Retention time: 12.2
Internal standard: 4'-hydroxymethamphetamine (6.0), methamphetamine (15.6)
Limit of quantitation: 920 ng/mL

OTHER SUBSTANCES

Extracted: 4'-hydroxyamphetamine, metabolites

KEY WORDS

SPE; rat

REFERENCE

Law,M.Y.L.; Moody,D.E. Simultaneous quantitation of amphetamine and 4'-hydroxyamphetamine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2029–2043.

SAMPLE

Matrix: urine

Sample preparation: Condition an Extra-Sep C18 SPE cartridge (Teknokroma) with 1 mL MeOH and 1 mL buffer. Adjust pH of 2 mL urine to ca. 10 with 100 μL concentrated ammonium hydroxide, add 5 μg β-phenylethylamine, add to the SPE cartridge, wash with 5 mL water, wash with 1 mL MeCN, elute with 2 mL MeOH. Add 100 μL EtOH:concentrated HCl 6:1 to the eluate, evaporate to dryness. Reconstitute with 1 mL buffer and 1 mL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, let stand at room temperature for 10 min, add 2 mL n-hexane:ethyl acetate 50:50, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 1% aqueous sodium bicarbonate adjusted to pH 10 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: Gradient. MeCN:0.5% propylamine in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 4.7
Internal standard: β-phenylethylamine (4.1)
Limit of detection: 4 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
SPE; derivatization

REFERENCE
Molíns Legua,C.; Campíns Falcó,P.; Sevillano Cabeza,A. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulfonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *672*, 81–88.

SAMPLE
Matrix: urine
Sample preparation: 100-300 μL Urine + 100 μL 1.5 M NaOH + 5 μg IS, make up to 1 mL with water, add to an Extrelut 1 SPE cartridge, let stand for 20 min, elute with 6 mL benzene (Caution! Benzene is a carcinogen!). Add the eluate to 1 mL 100 mM sulfuric acid, extract. Remove the aqueous layer and add it to 3 mL 1.5 M NaOH, add 5 μL benzoyl chloride, vortex vigorously, extract twice with 3 mL portions of n-hexane. Combine the organic layers and wash them twice with 3 mL portions of water, evaporate the organic to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Chiralcel OB-H
Mobile phase: n-Hexane:isopropanol 90:10
Column temperature: 55
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5 (L), 18 (D)
Internal standard: l-p-methoxyamphetamine (12)
Limit of detection: 30 ng

OTHER SUBSTANCES
Extracted: ethylamphetamine, methamphetamine

KEY WORDS
rat; derivatization; SPE; chiral

REFERENCE

Nagai,T.; Kamiyama,S.; Matsushima,K. Analysis of time-lapse changes of d- and l-enantiomers of racemic ethylamphetamine and stereoselective metabolism in rat urine by HPLC determination, *J.Anal.Toxicol.*, **1995**, *19*, 225–228.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)

Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)

Flow rate: A 1.25; B 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 9.2

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

KEY WORDS

column-switching

REFERENCE

Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 20-fold or more. 500 μL Diluted urine + 50 μL 500 ng/mL (+)-2,5-dimethoxyamphetamine hydrochloride + 100 μL 500 mM NaOH + 2 mL benzene (Caution! Benzene is a carcinogen!), shake for 15 min, centrifuge at 1200 g for 5 min. Remove 1.8 mL of the organic phase and add it to 220 μL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove 200 μL of the aqueous phase and add it to 40 μL 250 mM NaOH, add 50 μL 330 mM pH 7.8 phosphate buffer, add 250 μL MeCN, add 25 μL 3 mM (-)-1-(9-fluorenyl)ethyl chloroformate, let stand at room temperature for 24 h, add 30 μL 100 mM glycine in water, let stand for 30 min, add 750 μL pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness in a centrifugal evaporator at room temperature, reconstitute the residue in 300 μL MeCN:water 50:50, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Adsorbosphere HS C18

Mobile phase: MeCN:THF:20 mM pH 3.6 sodium acetate buffer 25:21:54

Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 37 (L), 40 (D)
Internal standard: (+)-2,5-dimethoxyamphetamine (33)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
rat; derivatization; chiral

REFERENCE
Sukbuntherng,J.; Hutchaleelaha,A.; Chow,H.-H.; Mayersohn,M. Separation and quantitation of the enantiomers of methamphetamine and its metabolites in urine by HPLC: Precolumn derivatization and fluorescence detection, *J.Anal.Toxicol.*, **1995**, *19*, 139–147.

SAMPLE
Matrix: urine
Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 1% pH 10 sodium bicarbonate buffer. 2 mL Urine + 400 μL 8% pH 10 sodium bicarbonate buffer, mix, centrifuge at 1500 g for 2 min, add a 2 mL aliquot of the supernatant to the SPE cartridge, wash with 3 mL water, pass 500 μL 2% sodium 1,2-naphthoquinone 4-sulfonate through the cartridge, pass 500 μL 1% pH 10 sodium bicarbonate buffer through the cartridge, let stand at room temperature for 15 min, wash with 3 mL water, elute with 1 mL MeCN:water 50:50, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:buffer from 40:60 to 50:50 over 2.5 min, to 70:30 over 0.5 min, maintain at 70:30 for 1.5 min, to 85:15 over 1 min, maintain at 85:15 for 1.5 min. (Buffer was 5 mL/L propylamine in water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.1
Internal standard: β-phenylethylamine (3.6)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine

KEY WORDS
derivatization; SPE

REFERENCE
Campíns-Falcó,P.; Sevillano-Cabeza,A.; Molíns-Legua,C.; Kohlmann,M. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with simultaneous sample clean-up and derivatization with 1,2-naphthoquinone 4-sulphonate on solid-phase cartridges, *J.Chromatogr.B*, **1996**, *687*, 239–246.

SAMPLE
Matrix: urine

Sample preparation: Inject 15 μL urine, inject a mixture of 5 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN and 45 μL water, and inject 10 μL buffer on to column A and elute to waste with mobile phase A. After 2.8 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. At the end of the run condition column A with 1 mL mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 PR-C18
Mobile phase: A water; B Gradient. MeCN:water from 40:60 to 70:30 over 15 min. to 100:0 over 5 min.
Flow rate: A 0.35; B 1
Injection volume: 15
Detector: F ex 264 em 313

CHROMATOGRAM

Retention time: 15.3
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: ephedrine, methamphetamine, norephedrine, 3-phenylpropylamine, pseudoephedrine

KEY WORDS

column-switching; derivatization; on-column derivatization

REFERENCE

Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of amphetamine and related compounds in urine using on-line derivatization in octadecyl silica columns with 9-fluorenylmethyl chloroformate and liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 69–78.

SAMPLE

Matrix: urine
Sample preparation: Inject 50 μL urine on to column A and elute to waste with mobile phase A, after 2 min inject a mixture of 25 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water and 25 μL buffer on to column A, stop the flow of mobile phase A, after 10 min start pump A, after 5 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. After each run flush column A with ethyl acetate for 1 min, n-hexane for 1 min, and ethyl acetate for 1 min, re-equilibrate with mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS C18
Mobile phase: A water; B Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 5
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: methamphetamine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herréz-Hernńdez,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 20 μL 100 μM 1-phenylethylamine in water + 400 μL concentrated HCl, heat at 80° for 1 h, cool, neutralize with 600 μL 25% ammonia, add 5 mL 10% sodium carbonate solution, add 2 mL 500 mM pH 10.5 sodium borate buffer, add 2 mL chloroform:isopropanol 75:25, vortex for 1 min, centrifuge at 12.5° at 1500 g for 10 min, repeat the extraction. Combine the organic layers and remove a 100 μL aliquot, add 10 μL acetic acid to the aliquot, evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL carbonate buffer, add 50 μL 10 mM fluorescein-4-isothiocyanate in EtOH, mix, heat in the dark at 80° for 15 min, inject a 20 μL aliquot. (Prepare carbonate buffer by adjusting the pH of 200 mM sodium bicarbonate to 9.0 with 200 mM sodium carbonate.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Daisopak SP-120-5-ODS (Daiso, Osaka)
Mobile phase: Gradient. MeCN:20 mM pH 7.9 sodium phosphate buffer 20:80 for 16 min then 24:76 (step-gradient).
Flow rate: 0.8
Injection volume: 20
Detector: F ex 496 em 518

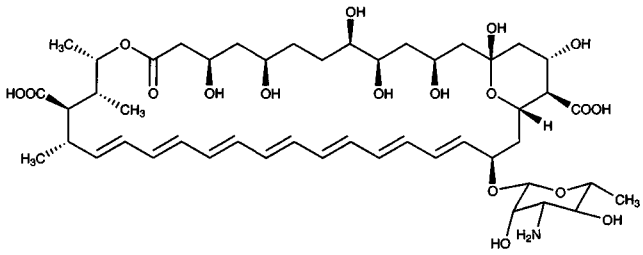
CHROMATOGRAM
Retention time: 32
Internal standard: 1-phenylethylamine (26.6)
Limit of detection: 5.5 nM

OTHER SUBSTANCES
Extracted: metabolites, methamphetamine, norepinephrine

KEY WORDS
derivatization

REFERENCE
Al-Dirbashi,O.; Kuroda,N.; Akiyama,S.; Nakashima,K. High-performance liquid chromatography of methamphetamine and its related compounds in human urine following derivatization with fluorescein isothiocyanate, *J.Chromatogr.B*, **1997**, *695*, 251–258.

Amphotericin



Molecular formula: $C_{47}H_{73}NO_{17}$
Molecular weight: 924.09
CAS Registry No.: 1397-89-3
Merck Index: 627

SAMPLE

Matrix: CSF
Sample preparation: Condition a BakerBond C18 SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 9 carbonate buffer. 1 mL CSF + 50 μL 10 μg/mL nystatin in MeOH, vortex briefly, add to the SPE cartridge, wash with 2 mL 100 mM pH 9 carbonate buffer, air dry for 2 min, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:10 mM pH 5 EDTA 35:65
Flow rate: 0.5
Injection volume: 100
Detector: UV 410

CHROMATOGRAM

Retention time: 7.6
Internal standard: nystatin (8.5)
Limit of detection: 0.5 ng/mL

KEY WORDS

dog; human; SPE; pharmacokinetics

REFERENCE

Liu,H.; Davoudi,H.; Last,T. Determination of Amphotericin B in cerebrospinal fluid by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1395–1400.

SAMPLE

Matrix: blood
Sample preparation: Add 800 μL cold MeOH to 200 μL serum, mix and centrifuge at 5° at 7000 rpm for 5 min. Filter (Millipore 0.45 μm) the supernatant, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 33 × 4.6 1.5 μm MICRA NPS RP-C18 (Micra Sccientific, Northbrook, IL, USA)
Mobile phase: MeCN:MeOH:50 mM sodium acetate 30:30:40
Flow rate: 0.7
Injection volume: 5
Detector: UV 382

CHROMATOGRAM

Retention time: 2.7
Limit of detection: 0.2 ng
Limit of quantitation: 0.625 ng

KEY WORDS
serum; dog; pharmacokinetics

REFERENCE
Betto,P.; Rajevic,M.; Bossù,E.; Gradoni,L. Improved assay for serum amphotericin-B by fast high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1857–1866.

SAMPLE
Matrix: blood
Sample preparation: Extract a 200-300 μL aliquot of whole blood with DMSO:MeOH 2:1 or chloroform:MeCN:DMSO 30:30:40 containing IS. Mix for 30 s, allow the mixture to sit for 1 hour and repeat mixing. Centrifuge, filter (0.45 μm) and inject a 50-100 μL aliquot of the supernatant. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water adjusted to pH 4.2 with EDTA 36:64
Flow rate: 1
Injection volume: 50-100
Detector: UV 405

CHROMATOGRAM
Internal standard: N-acetyl-amphotericin B
Limit of quantitation: 75 ng/mL

KEY WORDS
pharmacokinetics; whole blood

REFERENCE
Adedoyin,A.; Bernardo,J.F.; Swenson,C.E.; Bolsack,L.E.; Horwith,G.; DeWit,S.; Kelly,E.; Klasterksy,J.; Sculier,J.P.; DeValeriola,D.; Anaissie,E.; Lopez-Berestein,G.; Llanos-Cuentas,A.; Boyle,A.; Branch,R.A. Pharmacokinetic profile of ABELCET (amphotericin B lipid complex injection): combined experience from phase I and phase II studies, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2201–2208.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 600 μL MeOH, vortex, centrifuge at 10500 g for 5 min, inject an 80 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: MeCN:2.5 mM disodium EDTA 30:70
Flow rate: 1
Injection volume: 80
Detector: UV 405

CHROMATOGRAM
Retention time: 1.5
Limit of detection: 50 ng/mL

KEY WORDS
serum

REFERENCE
Lopez-Galera,R.; Pou-Clave,L.; Pascual-Mostaza,C. Determination of amphotericin B in human serum by liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 298–300.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize serum with MeOH, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 30 mm long C18
Mobile phase: MeCN:2.5 mM disodium EDTA 30:70
Flow rate: 1
Detector: UV 405

CHROMATOGRAM
Retention time: 1.5
Limit of quantitation: 50 ng/mL

KEY WORDS
pharmacokinetics; serum

REFERENCE
Ayestarán,A.; López,R.M.; Montoro,J.B.; Estíbalez,A.; Pou,L.; Julià,A.; López,A.; Pascual,B. Pharmacokinetics of conventional formulation versus fat emulsion formulation of amphotericin B in a group of patients with neutropenia, *Antimicrob.Agents Chemother.*, **1996**, *40*, 609–612.

SAMPLE
Matrix: blood, fibrin clot
Sample preparation: Digest fibrin clots with trypsin (1:1). 250 μL Serum or digested fibrin clot + 250 μL MeCN, vortex for 1 min, let stand at room temperature for 10 min, centrifuge at 2000 g, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 10 μm Spherisorb C18-ODS2
Mobile phase: MeOH:5 mM EDTA 70:30 adjusted to pH 7.8 with 1 M ammonium hydroxide
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 385

CHROMATOGRAM
Retention time: 4.7
Limit of quantitation: 10 ng/mL

KEY WORDS
rabbit; serum; human; pharmacokinetics

REFERENCE
Bouley,M.; Tod,M.; Chavanet,P.; Petitjean,O. The penetration of amphotericin B from an Intralipid formulation into fibrin loci in a rabbit model of candidiasis, *Biopharm.Drug Dispos.*, **1994**, *15*, 485–492.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 300 μL MeOH, heat in a 50° water bath for 15 min, cool at room temperature for 5 min, centrifuge at 9500 g for 10 min. Inject a 50 μL aliquot. Tissue. Mix 500 mg rat tissue with 4.5 mL MeOH and 500 μL 10 mM pH 7.4 phosphate buffer. Homogenize for 5 min, vortex for 5 min, centrifuge until the supernatant is clear, inject a 120 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS (plasma) or 216 × 4.6 10 μm C18 (Whatman) (tissue)
Mobile phase: MeOH:1 mM disodium EDTA containing 82 mM triethylamine and 96 mM phosphoric acid:deionized water 8:1:1.25 (plasma) or MeCN:10 mM sodium acetate 39.4:60.6
Column temperature: 35.5 (tissue)
Flow rate: 0.8 (plasma), 1 (tissue)
Injection volume: 50 (plasma), 120 (tissue)
Detector: UV 382

CHROMATOGRAM
Limit of quantitation: 50 ng/mL (plasma), 500 ng/g (tissue)

KEY WORDS
plasma; rat; pharmacokinetics; brain; kidney; liver; lung; spleen

REFERENCE
Boswell,G.W.; Bekersky,I.; Buell,D.; Hiles,R.; Walsh,T.J. Toxicological profile and pharmacokinetics of a unilamellar liposomal vesicle formulation of amphotericin B in rats, *Antimicrob.Agents Chemother.*, **1998**, *42*, 263–268.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 50-150 μL Serum + 50 μL 25 μg/mL IS in MeOH + 2 mL MeOH:acetic acid 90:10, vortex for 30 s, leave in the dark for 1 h, centrifuge at 1000 g for 10 min, decant, filter (0.45 μm), inject a 100 μL aliquot. Tissue. 100-200 mg Tissue + 50 μL 25 μg/mL IS + 500 μL 1 mM pH 7.4 phosphate buffer, vortex, homogenize using a manual glass homogenizer, add 2 mL MeOH:acetic acid 90:10, vortex for 30 s, leave in the dark for 1 h, centrifuge at 1000 g for 10 min, decant, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2 Alltech C18
Column: 300 × 3.9 10 μm μBondapak RP-C18
Mobile phase: MeCN:10 mM pH 4.0 acetate buffer 37:63
Flow rate: 1 for 6 min, then 2
Injection volume: 100
Detector: UV 383

CHROMATOGRAM
Retention time: 15
Internal standard: natamycin (6) (UV 303)
Limit of detection: 100 ng/mL
Limit of quantitation: 1 μg/mL

KEY WORDS
serum; lung; liver; mouse; pharmacokinetics

REFERENCE
Polikandritou Lambros,M.; Abbas,S.A.; Bourne,D.W.A. New high-performance liquid chromatographic method for amphotericin B analysis using an internal method, *J.Chromatogr.B*, **1996**, *685*, 135–140.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 0.5 g tissue with 1 mL MeOH for 1 min. 100 μL Serum or tissue homogenate + 100 μL cold MeCN, vortex for 10 s, centrifuge at 11800 g for 2 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco LC-1
Mobile phase: MeOH:5 mM EDTA buffer 65:35

CHROMATOGRAM
Limit of detection: 50 ng/mL

KEY WORDS
serum; rat; kidney; liver; lung

REFERENCE
Wasan,K.M.; Vadiei,K.; Lopez-Berestein,G.; Luke,D.R. Pharmacokinetics, tissue distribution, and toxicity of free and liposomal amphotericin B in diabetic rats, *J.Infect.Dis.*, **1990**, *161*, 562–566.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 0.5 g tissue in 4.5 mL MeOH. Extract tissue homogenate or 0.5 mL plasma using a 1 mL Bond-Elut C18 SPE cartridge.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:2.5 mM disodium EDTA 45:55
Detector: UV 382

CHROMATOGRAM
Limit of detection: ≤25 ng/g, ≤5 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics; liver; lung; kidney; spleen; heart; skin; lymph nodes; adrenal glands; thyroid; pancreas; testes; ileum; SPE

REFERENCE
Fielding,R.M.; Smith,P.C.; Wang,L.H.; Porter,J.; Guo,L.S. Comparative pharmacokinetics of amphotericin B after administration of a novel colloidal delivery system, ABCD, and a conventional formulation to rats, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1208–1213.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Condition a 1 mL 100 mg Bond-Elut C18 SPE cartridge with 1-2 mL MeOH, with 1-2 mL water, and with 3 mL 10 mM pH 7.4 phosphate buffer. 0.5 mL Blood or 0.3-0.5 g tissue + 0.5 mL 10 mM pH 7.4 phosphate buffer, homogenize (Polytron homogenizer) for 5-10 s, add 4 mL MeOH, vortex for 30 s, centrifuge at 2000 g for 10 min. Add 0.5 mL plasma, 1-2 mL urine, or 0.5-2 mL supernatant from blood or tissue to 4 mL 10 mM pH 7.4 phosphate buffer, add this mixture to the SPE cartridge at < 1 mL/min, wash with 3 mL MeOH:10 mM pH 7.4 phosphate buffer 40:60, centrifuge SPE cartridge at 2000 g for 2-3 min, elute with 0.75 (plasma) or 1 (others) mL MeCN:2.5 mM disodium EDTA 60:40 (plasma) or 50:50 (others), centrifuge to remove the last of the eluate, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:2.5 mM disodium EDTA 45:55
Flow rate: 1
Injection volume: 100
Detector: UV 382

CHROMATOGRAM

Retention time: 7

Limit of detection: ≤25 ng/mL (blood), 2.5 ng/mL (urine), 50 ng/g (tissue), ≤5 ng/mL (plasma)

KEY WORDS

plasma; SPE; rat; liver; kidney; lung; spleen; heart; brain; muscle; pharmacokinetics; stability

REFERENCE

Wang,L.H.; Smith,P.C.; Anderson,K.L.; Fielding,R.M. High-performance liquid chromatographic analysis of amphotericin B in plasma, blood, urine and tissues for pharmacokinetic and tissue distribution studies, *J.Chromatogr.*, **1992**, *579*, 259–268.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 100 μL Serum + 300 μL 160 ng/mL IS in ice-cold MeOH, vortex, centrifuge. Remove the supernatant and concentrate it under a stream of nitrogen at room temperature, reconstitute the residue in 150 μL MeOH, inject an aliquot. Urine. Dilute urine. 100 μL Diluted urine + 300 μL 160 ng/mL IS in ice-cold MeOH, vortex, inject an aliquot.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: MeCN:4 mM pH 7.0 Na_2HPO_4/KH_2PO_4 buffer 31:69

Flow rate: 1.2

Detector: UV 405

CHROMATOGRAM

Retention time: 3.5

Internal standard: 1-amino-4-nitronaphthalene (5.7)

Limit of quantitation: 22 ng/mL

KEY WORDS

rat; serum; protect from light; pharmacokinetics

REFERENCE

Chow,H.-H.; Wu,Y.; Mayersohn,M. Pharmacokinetics of amphotericin B in rats as a function of dose following constant-rate intravenous infusion, *Biopharm.Drug Dispos.*, **1995**, *16*, 461–473.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 *g* for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 346.2

CHROMATOGRAM
Retention time: 15.718

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bronchoalveolar lavage fluid
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with two 3 mL portions of MeCN and two 3 mL portions of 10 mM pH 7.4 sodium acetate buffer. Mix the total volume of bronchoaspiration or bronchoalveolar lavage fluid with an equal volume of MeOH for 1 min, centrifuge at 3000 rpm for 20 min, combine the supernatant with an equal volume (?) of 10 mM pH 7.4 sodium acetate buffer, add to the SPE cartridge, wash five times with 3 mL portions of MeOH:10 mM pH 7.4 sodium acetate buffer 50:50, elute with two 1.5 mL portions of MeOH. Evaporate the eluate to dryness under nitrogen, reconstitute the residue in 400 μL MeOH, vortex for 15 s, inject an aliquot.

HPLC VARIABLES
Column: 30 × 4.6 3 μm Perkin-Elmer ODS
Mobile phase: MeCN:2.5 mM EDTA disodium dihydrate 30:70
Flow rate: 1
Injection volume: 80
Detector: UV 405

CHROMATOGRAM
Retention time: 1.5
Limit of detection: 50 ng/mL

KEY WORDS
SPE

REFERENCE
Lopez,R.; Pou,L.; Andres,I.; Monforte,V.; Roman,A.; Pascual,C. Amphotericin B determination in respiratory secretions by reversed-phase liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 135–139.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL samples to 5 or 10 mL with 5% dextrose, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Beckman ODS C18
Mobile phase: MeCN:MeOH:buffer 37:18:45 (Buffer was 50 mM sodium acetate and 3 mM disodium EDTA adjusted to pH 5.0 with glacial acetic acid.)
Flow rate: 1.2
Injection volume: 20

Detector: UV 405

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 625 ng/mL

OTHER SUBSTANCES
Simultaneous: amphotericin A, amphotericin X

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Kintzel,P.E.; Kennedy,P.E. Stability of amphotericin B in 5% dextrose injection at concentrations used for administration through a central venous line, *Am.J.Hosp.Pharm.*, **1991**, *48*, 283–285.

SAMPLE
Matrix: formulations
Sample preparation: Directly inject a 20 μL aliquot.

HPLC VARIABLES
Column: 10 μm Radial-Pak μBondapak C18
Mobile phase: MeOH:MeCN:2.5 mM EDTA 50:35:20
Flow rate: 1.8
Injection volume: 20
Detector: UV 405

CHROMATOGRAM
Retention time: 5.8

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Mitrano,F.P.; Outman,W.R.; Baptista,R.J.; Palombo,J.D. Chemical and visual stability of amphotericin B in 5% dextrose injection stored at 4 degrees C for 35 days, *Am.J.Hosp.Pharm.*, **1991**, *48*, 2635–2637.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Liposomal preparation + 100 μL MeOH, vortex for 10 s, centrifuge at 13000 g for 2 min, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco LC-1
Mobile phase: MeOH:0.005% EDTA 65:35
Flow rate: 2
Injection volume: 75
Detector: UV 405

CHROMATOGRAM
Limit of detection: 50 ng/mL

KEY WORDS
liposomal preparations

REFERENCE

Wasan,K.M.; Morton,R.E.; Rosenblum,M.G.; Lopez-Berestein,G. Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high-density lipoproteins: Role of lipid transfer protein, *J.Pharm.Sci.*, **1994**, *83*, 1006–1010.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in DMSO to 10 mg/mL, dilute 1:20 with MeOH.

HPLC VARIABLES

Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:50 mM phosphate buffer (pH 3.5-8.1) 30:70 to 35:65
Flow rate: 0.4-2
Detector: UV 313

OTHER SUBSTANCES

Simultaneous: nystatin

KEY WORDS

for amphotericin A

REFERENCE

Aszalos,A.; Bax,A.; Burlinson,N.; Roller,P.; McNeal,C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A, *J.Antibiot.(Tokyo)*, **1985**, *38*, 1699–1713.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 15 mM, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 3 μm Econosphere C18
Mobile phase: MeOH:buffer 65:35 (Buffer was 6.3 g NaH_2PO_4 in 1 L water, adjust pH to 2.6 with phosphoric acid.)
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 407

CHROMATOGRAM

Retention time: 10

REFERENCE

Backes,B.J.; Rychnovsky,S.D. A reverse-phase HPLC assay for measuring the interaction of polyene macrolide antifungal agents with sterols, *Anal.Biochem.*, **1992**, *205*, 96–99.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: C18
Mobile phase: MeOH:DMF 20:80
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

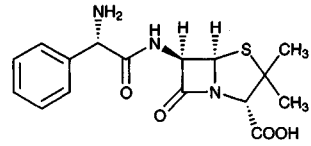
CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: cefpirome

KEY WORDS
stability-indicating; protect from light

REFERENCE
Allen,L.V.,Jr.; Stiles,M.L.; Prince,S.J.; Sylvestri,M.F. Stability of cefpirome sulfate in the presence of commonly used intensive care drugs during simulated Y-site injection, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2427–2433.

Ampicillin



Molecular formula: $C_{16}H_{19}N_3O_4S$
Molecular weight: 349.41
CAS Registry No.: 69-53-4 (anhydrous), 32388-53-7 (monohydrate), 23277-71-6 (K salt), 7177-48-2 (trihydrate), 69-52-3 (Na salt)
Merck Index: 628
Lednicer No.: 1 413, 2 437, 4 179

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Plasma. 50 μL Plasma + 50 μL IS solution + 50 μL MeCN, mix for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Urine. 100 μL IS solution + 200 μL MeCN + 100 μL urine, mix for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot. Tissue. Weight out finely chopped tissue and suspend it in 200 μL water. Add 100 μL 100 μg/mL IS, sonicate for 60 s. Add 200 μL MeCN, vortex for 30 s, centrifuge at 10000 g for 15 min. Inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard C18 (Alltech)
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:50 mM pH 5.0 sodium dihydrogen phosphate 10:90
Flow rate: 1.0
Detector: UV 215

CHROMATOGRAM

Retention time: 4.3
Internal standard: cefotaxime (11.6)
Limit of quantitation: 500 ng/mL (plasma), 1 μg/mL (urine), 2.5 μg/g (tissue)

KEY WORDS

plasma; muscle; rat; pharmacokinetics

REFERENCE

Cross,S.E.; Thompson,M.J.; Roberts,M.S. Distribution of systemically administered ampicillin, benzylpenicillin, and flucloxacillin in excisional wounds in diabetic and normal rats and effects of local topical vasodilator treatment, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1703–1710.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.827

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Capsules. Dissolve the powder from five capsules and the capsule shells in water, dilute to 1 L with water, filter an aliquot of the solution (0.2 μm), dilute 10-fold with water, inject an aliquot. Syrup. Reconstitute the syrup powder, dilute 200-fold with water, filter, inject an aliquot. Neonatal suspension. Reconstitute the powder, dilute 800-fold with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 100 × 2.0 3 μm Hypersil ODS
Mobile phase: MeCN:20 mM phosphate buffer 15:85 containing 100 mM sodium dodecyl sulfate, adjusted to pH 2.0 with orthophosphoric acid
Flow rate: 0.4
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: degradation products, cloxacillin

KEY WORDS
capsules; suspensions; syrup

REFERENCE
Shakoor,O.; Taylor,R.B. Analysis of ampicillin, cloxacillin and their related substances in capsules, syrups and suspensions by high-performance liquid chromatography, *Analyst*, **1996**, *121*, 1473–1477.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 18.5 min), evaporate to <1 mL under reduced pressure, add 200 μL 10 mM KH_2PO_4 containing 10 mM phosphoric acid and 10 mM

sodium decanesulfonate, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM phosphoric acid containing 5 mM potassium dihydrogen phosphate and 5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg Baker-10 C18 SPE cartridge (J.T. Baker) with 3 mL MeOH and 3 mL distilled water. Add 20 mL MeCN to 10 mL milk, vortex for 1 min, centrifuge at 1500 g for 10 min, concentrate the supernatant to 2-3 mL on a rotary evaporator at 40°, add to the SPE cartridge, dry the cartridge under reduced pressure for 3 min, elute with 1 mL MeOH, filter (0.45 μm) the eluate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Kaseisorb LC ODS-300-5 (Tokyo Kasei)
Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 buffer 20:10:80 containing 5 mM sodium 1-decanesulfonate, adjusted to pH 3.5 with concentrated phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, nafcillin, penicillin G

KEY WORDS
SPE

REFERENCE
Takeba,K.; Fujinuma,K.; Miyazaki,T.; Nakazawa,H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 205–211.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl, do not allow to go dry. 5 mL Milk + 500 μL 30% trichloroacetic acid, vortex thoroughly for 10 s, centrifuge at 0° at 3300 g for 30 min. Remove the liquid supernatant and add it to 100 μL 4 M NaOH (to pH 5.2), vortex for 10 s, add 1 mL 20% NaCl, vortex for 10 s, add to the SPE cartridge at 3 mL/min, wash with 1 mL 2% NaCl, wash with 1 mL water, elute with 1 mL MeCN:100 mM pH 6.5 phosphate buffer 40:60. Add 20 μL 2 M NaOH to the eluate and vortex for 10 s (pH 8), add 10 μL 200 mM acetic anhydride in MeCN, let stand for 3 min, add 500 μL reagent,

vortex, heat at 65° for 10 min, cool to room temperature, inject a 200 μL aliquot. (Prepare reagent by dissolving 13.78 g 1,2,4-triazole in 60 mL water, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 4 M NaOH, make up to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:buffer 18:12:70 (Prepare buffer by dissolving 4.969 g NaH_2PO_4, 10.139 g $Na_2HPO_4.2H_2O$, 3.894 g sodium thiosulfate pentahydrate, and 6.791 g tetrabutylammonium hydrogen sulfate in 800 mL water, make up to 1 L with water.)
Flow rate: 0.8
Injection volume: 200
Detector: UV 325

CHROMATOGRAM

Retention time: 16
Limit of detection: 3 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin
Noninterfering: cloxacillin, oxacillin, penicillin G

KEY WORDS

derivatization; SPE; cow

REFERENCE

Verdon,E.; Couedor,P. Determination of ampicillin residues in milk by ion-pair reversed phase high performance liquid chromatography after precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1201–1207.

SAMPLE

Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH,

make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM
Retention time: 32.5
Internal standard: penicillin V (28.5)
Limit of detection: 1.5 ng/mL
Limit of quantitation: 2.2 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin, cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS
derivatization; cow; SPE

REFERENCE
Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE
Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 30:70 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 5.6
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:1 M acetic acid:1 M KH_2PO_4:water 8:0.1:1:90.9
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Isolute SCX SPE cartridge (Jones Chromatography, Hengoed, UK) with MeOH and water. Condition a 100 mg PGC (porous graphitic carbon) SPE cartridge (Shandon, Runcorn, UK) with acetone and pH 7.7 borate buffer. Add 20 mL water to 5 g tissue, homogenize, add 5 mL 170 mM sulfuric acid and 5 mL 5% aqueous sodium tungstate, mix well, centrifuge at 14000 g for 5 min. Discard pellet, add six drops orthophosphoric acid to the supernatant to adjust the pH to 2-2.5. Add it to the SCX SPE cartridge, allow to flow through the cartridge under vacuum at 2 mL/min, wash with 5 mL 10 mM sulfuric acid, elute with 10 mL pH 7.7 borate buffer. Add the eluate to the PGC SPE cartridge. Wash with 5 mL water, place in-line filter (0.2 μm, Anotop) below cartridge, elute with 20 mL acetone. Evaporate to dryness. Add 500 μL water to the dry residue, add 20 μL 2% acetic anhydride in MeCN and let stand for 3 min. Add 500 μL triazole/mercuric chloride derivatizing reagent and heat the mixture at 65° for 20 min. Inject a 100 μL aliquot. (Borate buffer was 200 mM boric acid adjusted to pH 7.7 with 40% NaOH solution. Triazole/mercuric chloride reagent was prepared by mixing 34.45 g 1,2,4-triazole with 150 mL water and 25 mL 10 mM mercuric chloride, adjusted to pH 9.0 with 1 M NaOH and made up to 250 mL with water.) GL –5 μm Kromasil KR 100 C8 (Hichrom)

HPLC VARIABLES
Column: 250 × 3.2 5 μm Kromasil KR 100 C8 (Hichrom)
Mobile phase: MeCN:buffer 20:80 (Buffer was 15 mM potassium dihydrogen phosphate and 15 mM sodium thiosulfate)
Flow rate: 0.55
Injection volume: 100
Detector: UV 325

CHROMATOGRAM
Retention time: 24.5
Limit of detection: 5 μg/kg (muscle)
Limit of quantitation: 50 μg/kg (muscle), 100 μg/kg (liver)

OTHER SUBSTANCES
Extracted: amoxicillin

KEY WORDS
SPE; cow; liver; muscle; derivatization

REFERENCE
Rose,M.D.; Tarbin,J.; Farrington,W.H.; Shearer,G. Determination of penicillins in animal tissues at trace residue concentrations: II. Determination of amoxicillin and ampicillin in liver and muscle using cation exchange and porous graphitic carbon solid phase extraction and high-performance liquid chromatography, *Food Addit.Contam.*, **1997**, *14*, 127–133.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize (Ultra-turrax T25) 5 g muscle with 14 mL 10 mM pH 4.5 sodium phosphate buffer at 10000 rpm for 2 min, add 1 mL 75% trichloroacetic acid in water, shake vigorously for 30 s, centrifuge at 3500 g for 10 min, filter (paper) the supernatant. Remove a 1 mL aliquot of the filtrate, add 200 μL 20% trichloroacetic acid in water, add 200 μL 7% formaldehyde in water, vortex for 20 s, heat at 100° for 30 min, cool to room temperature, make up to 2 mL with MeCN:water 20:80, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Prodigy ODS-3 (Phenomenex)
Mobile phase: MeCN:20 mM pH 3.5 KH_2PO_4 buffer 25:75
Flow rate: 1
Injection volume: 100
Detector: F ex 346 em 422

CHROMATOGRAM

Retention time: 12.5
Limit of detection: 0.6 ng/g
Limit of quantitation: 1.5 ng/g

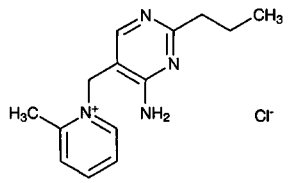
KEY WORDS

derivatization; muscle; cow; pig; chicken; fish; catfish

REFERENCE

Luo,W.; Ang,C.Y.W.; Thompson,H.C.,Jr. Rapid method for the determination of ampicillin residues in animal muscle tissues by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *694*, 401–407.

Amprolium



Molecular formula: $C_{14}H_{19}ClN_4$
Molecular weight: 278.78
CAS Registry No.: 121-25-5
Merck Index: 631
Lednicer No.: 1 264

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 100 ng/mL IS in 330 mM perchloric acid + 500 μL 330 mM perchloric acid, vortex for 30 s, centrifuge at 2150 g for 10 min. Remove the supernatant and allow it to stand for 3 h, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Sumipax PG-ODS-filter (Sumika, Osaka)
Column: 250 × 4.6 5 μm Capcell pack C18 UG-120 (Shiseido, Tokyo)
Mobile phase: MeCN:200 mM KH_2PO_4 10:90 containing 5 mM sodium 1-hexanesulfonate
Column temperature: 40
Flow rate: 0.6
Injection volume: 30
Detector: F ex 400 em 460 following post-column reaction. The column effluent mixed with the reagent pumped at 0.6 mL/min and the mixture flowed through a 10 m × 0.25 mm ID stainless steel coil at 40° to the detector. (Prepare reagent by dissolving 50 g NaOH and 800 mg potassium ferricyanide in 1 L water, store in the dark, discard after 24 h.)

CHROMATOGRAM

Retention time: 13
Internal standard: beclotiamine (3-[(4-amino-2-methyl-5-pyrimidinyl)methyl-5-(2-chloroethyl)-4-methylthiazolium chloride, Sankyo, Tokyo) (12)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, thiamine
Noninterfering: ethopabate

KEY WORDS

post-column reaction; chicken; plasma; pharmacokinetics

REFERENCE

Hamamoto,K.; Koike,R.; Shirakura,A.; Sasaki,N.; Machida,Y. Rapid and sensitive determination of amprolium in chicken plasma by high-performance liquid chromatography with post-column reaction, *J.Chromatogr.B*, **1997**, *693*, 489–492.

SAMPLE

Matrix: blood, tissue
Sample preparation: Slurry 6 g alumina (alumina B Akt. I, ICN Biomedicals) in MeCN:MeOH 60:40, add to a 300 × 15 column, wash with 30 mL MeCN:MeOH 60:40. Homogenize (Niti-on Bio-mixer BM-2) 5 g chopped tissue or plasma with 25 mL MeCN for 2 min, wash twice with 20 mL portions of MeCN, filter (cotton plug), wash filter with 30 mL n-hexane saturated with MeCN, add 30 g anhydrous sodium sulfate to the filtrate, let stand at room temperature for 30 min, filter (cotton plug), add 30 mL isopropanol to the filtrate. Evaporate the filtrate to dryness at 35°, reconstitute with 5 mL MeCN:MeOH 60:40, sonicate, add to the column, elute with 35 mL MeCN:MeOH 60:40. Add 10 mL

isopropanol to the eluate and evaporate it to dryness at 40°, reconstitute with 1 μg/mL chloramphenicol in mobile phase, filter (Gelman Ekikurodisk 13 CR), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 L-column ODS (Chemicals Inspection and Testing Institute, Tokyo)
Mobile phase: MeCN:200 mM KH_2PO_4 15:85 containing 5 mM sodium 1-hexanesulfonate
Column temperature: 40
Flow rate: 0.7
Injection volume: 20
Detector: F ex 367 em 470 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and the mixture flowed through a 10 m × 0.25 mm ID stainless steel coil at 40° to the detector. (Prepare reagent by dissolving 50 g NaOH in water, adding 800 mg potassium ferricyanide, and making up to 1 L with water.)

CHROMATOGRAM
Retention time: 8
Limit of detection: 2-4 ng/g

KEY WORDS
post-column reaction; chicken; muscle; liver; kidney; skin; plasma; SPE

REFERENCE
Takahashi,Y.; Sekiya,T.; Nishikawa,M.; Endoh,Y.S. Simultaneous high-performance liquid chromatographic determination of amprolium, ethopabate, sulfaquinoxaline, and N4-acetylsulfaquinoxaline in chicken tissues, *J.Liq.Chromatogr.*, **1994**, *17*, 4489–4512.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Mix egg yolk with an equal amount of water, homogenize (Ultraturrax) for 30 s. Blend (Lameris Lab Blender 400 stomacher) 20-30 g tissue with twice the amount of water for 5 min, centrifuge at 460 g for 10 min. Dialyze (24" dialyzer with membrane Type C (Technicon, Tarrytown NY)) diluted egg yolk or tissue supernatant against water (both pumped at 0.6 mL/min), inject a 2 mL aliquot of the dialysate onto column A at 1 mL/min, wash with water at 1 mL/min for 6 min, backflush the contents of column A onto column B with mobile phase, after 2 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 4.6 37-50 μm Corasil C18; B 20 mm long LC8 (Supelco) + 150 × 4.6 5 μm Supelcosil LC8-DB
Mobile phase: MeOH:water:acetic acid:triethylamine 25:75:1:0.5 containing 5 mM heptanesulfonate
Column temperature: 40
Flow rate: 1
Injection volume: 2000
Detector: F ex 365 em 470 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.5 mm ID knitted PTFE coil to the detector. (Prepare reagent by dissolving 25 g NaOH and 160 mg potassium ferricyanide in 100 mL water.)

CHROMATOGRAM
Retention time: 10
Limit of detection: 3 ng/g

KEY WORDS
post-column reaction; column-switching; dialysis; yolk; muscle; chicken

REFERENCE

van Leeuwen,W.; Wilhelmus van Gend,H. Determination of amprolium in egg yolk and muscle tissue (chicken) by HPLC with post-column reaction and fluorometric detection, using on-line sample clean-up and pre-concentration steps, *Z.Lebensm.Unters.Forsch.*, **1988**, *186*, 500–504.

SAMPLE

Matrix: feed, premix

Sample preparation: Feed. 1 g Feed + 2 mL 5 μg/mL thiamine monophosphate + 10 mL 5% sulfosalicylic acid + 10 mL hexane, vortex for 1 min, centrifuge at 2400 g for 10 min. Remove the aqueous layer and filter it (0.45 μm, Gelman Acro LC 13). Inject a 360 μL aliquot onto a 300 × 6 glass column packed with 200-400 mesh Dowex AG 2-X8 anion exchange resin, elute with 100 mM HCl at 1.2 mL/min, after about 2 min collect a 6-10 mL fraction, neutralize with 1 M NaOH (pH 7.0 ± 0.5), inject a 500 μL aliquot of this fraction (J.Agric.Food Chem. 1980, 28, 1145). After 15-20 samples clean the sulfosalicylic acid from the column by backflushing with 700 mM NaCl containing 100 mM HCl and 2% ferric chloride at 1.2 mL/min, flush for 20-30 min after the last of the iron chelate has gone (about 3 h). Re-equilibrate for 30 min. Premix. 0.8 mg Premix + 0.1 mg pyrithiamine + 10 mL water, grind (Omni-Mixer), add 10 mL hexane, grind for 5 min, centrifuge at 2400 g at 4° for 10 min. Filter the aqueous layer (0.45 μm, Millipore), dilute 20 times, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Rainin RP-18 guard column

Column: 30 × 3 3 μm Perkin-Elmer C18

Mobile phase: Feed. Gradient. 100 mM pH 5.5 sodium phosphate buffer for 6 min then 100 mM pH 2.6 sodium phosphate buffer for 19 min, re-equilibrate with original buffer for 15 min. Premix. Isocratic. 100 mM pH 2.6 Sodium phosphate buffer.

Flow rate: 1

Injection volume: 10

Detector: F ex 339 em 432, following post-column reaction with 0.01% potassium ferricyanide in 15% NaOH pumped at 1 mL/min. The reagent is mixed with the column effluent and passed through a 7 m × 0.4 mm i.d. PTFE tube, kept at 32°, to the detector.

CHROMATOGRAM

Retention time: 13.98 (feed), 4.03 (premix)

Internal standard: thiamine monophosphate (5.26), pyrithiamine (1.62)

OTHER SUBSTANCES

Simultaneous: thiamine diphosphate, thiamine

REFERENCE

Vanderslice,J.T.; Huang,M.-H.A. Liquid chromatographic determination of amprolium in poultry feed and premixes using postcolumn chemistry with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 920–922.

SAMPLE

Matrix: feed, premix

Sample preparation: Weigh out 4-12 g feed, add 100 mL MeOH:water 2:1 containing 5 mM sodium dioctylsulfosuccinate and 10 mM calcium chloride, shake mechanically for 1 h, centrifuge, force 8-10 mL solution through a Sep-Pak alumina A SPE cartridge, discard first 3 mL effluent, collect next 4-6 mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 mm long 5 μm Econosphere C18 or 250 × 4.6 10 μm Whatman ODS-3

Mobile phase: MeCN:water 40:60 containing 4 mM sodium dioctylsulfosuccinate, 0.3% diethylamine, and 1% acetic acid

Flow rate: 1.5

Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Noninterfering: ethopabate, sulfonamides, arsanilic acid, penicillin, streptomycin, chlortetracycline

KEY WORDS
rugged; SPE

REFERENCE
Kentzer,E.J.; Cottingham,L.S.; Smallidge,R.L. Ion-pair reverse-phase liquid chromatographic determination of amprolium in complete feeds and premixes, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 251–255.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax TP 18/10) 3 g tissue with 1 mL water and 4 mL acetone for 6 s, centrifuge at 5000 rpm for 3 min. Transfer 4 mL supernatant, add 5 mL dichloromethane, mix for 5 s, centrifuge at 3000 rpm for 3 min. Transfer the upper (water) layer to another tube and add 1 g NaCl, 3 mL MeCN, and 1 mL 300 mM NaOH. Shake vigorously for 20 s, centrifuge at 3000 rpm for 2 min, transfer the upper layer to another tube. Extract the remainder twice with 3 mL MeCN, discard the water layer. Evaporate the MeCN layers to dryness at 60° under a stream of nitrogen. Dissolve residue in 500 μL 20 mM KH_2PO_4 and filter (Spin-X) while centrifuging at 5600 g for 3 min.. Inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:buffer 10:90 (Prepare buffer by dissolving 27.2 g KH_2PO_4 and 0.94 g hexane sulfonic acid sodium salt in 750 mL water, make up to 1 L with water.)
Flow rate: 0.8
Injection volume: 20
Detector: F ex 365 em 470 following post-column reaction. The column effluent mixed in a vortex mixer with reagent pumped at 0.7 mL/min and this mixture flowed through a 10 m × 0.3 mm ID PTFE coil illuminated in a Beam Boost Photochemical Reactor to the detector. (The reagent was 1.25 M NaOH containing 25 mM potassium ferricyanide.)

CHROMATOGRAM
Retention time: 5.2
Limit of quantitation: 5 ng/g

KEY WORDS
chicken; muscle; post-column reaction; post-column photochemical derivatization

REFERENCE
Hormazabal,V.; Yndestad,M. Rapid assay for the determination of residues of amprolium and ethopabate in chicken meat by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2517–2525.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a cleanup column by plugging a 10 mm i.d. column with glass wool and adding 5 g activity I alumina, prewash with 70 mL MeCN:MeOH 90:10.

Homogenize 10 g minced chicken muscle with 50 mL MeOH at maximum speed (Ultra-Turrax T-18), filter through cotton, repeat extraction with another 50 mL MeOH. Combine filtrates, add 20 mL 1-propanol, concentrate to 3-4 mL under vacuum at 45°. Add the residue to 20 mL MeCN and 50 mL n-hexane, shake vigorously by hand for 5 s, discard n-hexane layer, add another 50 mL n-hexane, shake for 5 min on a mechanical shaker, discard n-hexane layer. Evaporate the lower phase to dryness under vacuum at 45°, dissolve the residue in 1 mL MeOH, add to cleanup column, wash with 30 mL MeCN:MeOH 90:10, elute with 30 mL MeCN:water 95:5. Evaporate the eluate to dryness under vacuum at 45°, reconstitute the residue in 1 mL water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 7 μm LiChrosorb RP-8
Mobile phase: MeCN:200 mM KH_2PO_4 20:80 containing 5 mM sodium 1-hexanesulfonate
Column temperature: 30
Flow rate: 0.7
Injection volume: 10
Detector: F ex 367 em 470 following post-column reaction with 25 g NaOH and 0.4 g potassium ferricyanide in 500 mL water pumped at 0.7 mL/min. The column effluent and reagent were mixed and flowed through a 3 m × 0.3 mm i.d. stainless steel reaction coil to the detector.

CHROMATOGRAM

Retention time: 10
Limit of detection: 0.01 ppm

OTHER SUBSTANCES

Noninterfering: ethopabate, sulfonamides

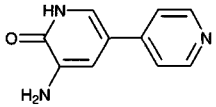
KEY WORDS

chicken; muscle; SPE

REFERENCE

Nagata,T.; Saeki,M. Liquid chromatographic determination of amprolium in chicken tissues, using post-column reaction and fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 941–943.

Amrinone



Molecular formula: $C_{10}H_9N_3O$
Molecular weight: 187.20
CAS Registry No.: 60719-84-8
Merck Index: 634
Lednicer No.: 3 147; 4 90, 115, 163

SAMPLE

Matrix: blood
Sample preparation: Mix plasma or serum (minimum 50 μL) with an equal volume MeCN, vortex for 15 s. Incubate at ambient temperature for 15 min, vortex for 15 s, centrifuge at 39 000 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 2.1 5 μm C18 narrow-bore (Hewlett-Packard)
Mobile phase: Gradient. A was MeCN. B was 100 mm pH 6.0 phosphate buffer. A:B from 5:95 to 10:90 over 13.5 min. (Buffer was prepared by mixing 2 L 100 mm monobasic sodium phosphate with 280 mL 100 mM dibasic sodium phosphate.)
Flow rate: 0.4
Injection volume: 25
Detector: UV 320, UV 345

CHROMATOGRAM

Retention time: 3.7
Limit of detection: 100 μg/L (320 nm), 500 μg/L (345 nm)

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, bumetanide, carbamazepine, cefamandole, cefonicid, cefotaxime, cefoxitin, cefprozil, ceftazidime, ceftriaxone, ceftibuten, ceftizoxime, cefuroxime, cephalexin, cephalothin, desipramine, diazepam, digoxin, dobutamine, dopamine, doxepin, epinephrine, ethosuximide, fentanyl, furosemide, gentamicin, imipramine, methotrexate, midazolam, morphine, norepinephrine, phenobarbital, phenytoin, primidone, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin (Monitoring at 345 nm may be necessary to avoid interference by cephalosporins.)

KEY WORDS

plasma; serum

REFERENCE

Pappas,J.B.; Allen,E.M.; Ross,M.; Banner,W.,Jr. HPLC micromethod for amrinone and metabolites in patients receiving concurrent cephalosporin therapy, *Clin.Chem.*, **1996**, *42*, 761–765.

SAMPLE

Matrix: blood
Sample preparation: Extract serum.

HPLC VARIABLES

Column: 150 × 3.3 7 μm Separon SGX
Mobile phase: 80 mM ammonium perchlorate in MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.89

OTHER SUBSTANCES
Simultaneous: nicotine, strychnine, neostigmine

KEY WORDS
serum

REFERENCE
Eigendorf,H.G.; Nagel,S. Zur Analytik von Amrinone (Cordemcura). 2. Mitteillung: Hochdruckglüssig-chromatographie [The analysis of amrinone (Cordemcura). 2. High pressure liquid chromatography], *Pharmazie*, **1987**, *42*, 631–631.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL ethyl acetate, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic phase and evaporate it under nitrogen at 30°, reconstitute the residue in 100 μL MeCN:5 mM pH 3.2 phosphate buffer 1:1, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Guard-pak cyano (Waters)
Column: 100 × 8 4 μm Nova Pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 4.18
Limit of quantitation: 250 ng/mL

KEY WORDS
plasma

REFERENCE
Bansal,R.; Louridas,A.T.; Gottesman,R.D.; Aranda,J.V. Determination of amrinone in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Liq.Chromatogr.*, **1994**, *17*, 3531–3539.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS III C18
Mobile phase: MeOH:water:0.5 M borate 40:58:2, pH 7.0
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3

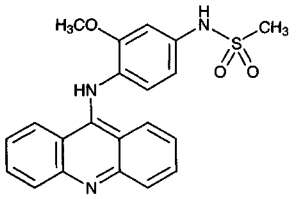
OTHER SUBSTANCES
Noninterfering: digoxin

KEY WORDS
injections; stability-indicating; 5% dextrose; 0.45% NaCl

REFERENCE

Riley,C.M.; Junkin,P. Stability of amrinone and digoxin, procainamide hydrochloride, propranolol hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures, *Am.J.Hosp.Pharm.*, **1991**, *48*, 1245–1252.

Amsacrine



Molecular formula: $C_{21}H_{19}N_3O_3S$
Molecular weight: 393.47
CAS Registry No.: 51264-14-3
Merck Index: 635

SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 20 µM IS in MeOH to a glass tube and evaporate the MeOH to dryness under a stream of nitrogen at 35°, add 500 µL plasma, adjust pH to 3.0-4.0 with 120 µL 500 mM HCl, vortex gently, add 5 mL hexane, shake for 20 min, centrifuge at 1720 g for 10 min. Remove the aqueous layer and adjust the pH to 9.0 with 500 µL saturated sodium tetraborate, add 6 mL diethyl ether, shake for 15 min, centrifuge at 1720 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 µL MeOH, inject a 20-40 µL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 µm Radial-Pak C18 (Waters)
Mobile phase: MeCN:water:buffer 396:594:10 (Buffer was 13.9 mL triethylamine in 60 mL water, adjust pH to 3.0 with phosphoric acid, dilute to 100 mL with water.)
Flow rate: 7
Injection volume: 20-40
Detector: UV 254

CHROMATOGRAM

Retention time: 3.4
Internal standard: 4'-(3-methyl-9-acridinylamino)methanesulfonanilide (4.3)
Limit of detection: 50 nM

OTHER SUBSTANCES

Noninterfering: doxorubicin, chlorambucil, cytosine arabinoside, 5-fluorouracil, lomustine, melphalan, methotrexate, prednisolone, 6-thioguanine, vincristine, vinblastine, degradation products

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Jurlina,J.L.; Paxton,J.W. High-performance liquid-chromatographic method for the determination of 4'-(9-acridinylamino)methanesulfon-m-anisidide in plasma, *J.Chromatogr.*, **1983**, *276*, 367–374.

SAMPLE

Matrix: blood, cells
Sample preparation: 500 µL Plasma, whole blood, or cells + 100 µL 5 µg/mL IS in 5% dextrose, adjust pH to 3.0-4.0 with 500 mM HCl, extract with hexane. Remove the aqueous layer and adjust the pH to 9.0 with saturated sodium tetraborate, extract with anhydrous diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 200 µL MeOH:148 mM phosphoric acid 50:10, centrifuge at 6000 g for 10 min, inject an aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 LiChrosorb RP-8
Column: 150 × 4.6 8 µm Cp-Spher C8 (Chrompack)

Mobile phase: MeCN:water:buffer 396:594:10 (Buffer was 13.9 mL triethylamine in 60 mL water, adjust pH to 3.0 with 85% phosphoric acid, dilute to 100 mL with water.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 2.8
Internal standard: N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide (CI-921) (3.5)
Limit of detection: 6 ng/mL

KEY WORDS
whole blood; plasma; pharmacokinetics

REFERENCE
Brons,P.P.T.; Wessels,J.M.C.; Linssen,P.C.M.; Haanen,C.; Speth,P.A.J. Determination of amsacrine in human nucleated hematopoietic cells, *J.Chromatogr.*, **1987**, *422*, 175–185.

SAMPLE
Matrix: tissue
Sample preparation: 1 g Tissue + 1 mL saline, mince, homogenize (Polytron PT-10) at 27000 rpm for 10-15 min, adjust pH to 2.0 with 500 mM HCl, centrifuge at 12000 g for 10 min. Add the supernatant to six volumes n-hexane, mix thoroughly. Remove the aqueous phase and adjust its pH to 9.0 with saturated sodium borate, extract with six volumes of ethyl acetate. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water:5% pH 4.3 sodium phosphate 450:50:3
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 10-12
Limit of detection: 200 ng/g

KEY WORDS
bladder; liver; lymph node; kidney; adrenal; ovary; stomach; thyroid; heart; lung; testicle; muscle; fat; spleen; pancreas; colon; prostate; brain; oncocytoma

REFERENCE
Stewart,D.J.; Zhengang,G.; Lu,K.; Savaraj,N.; Feun,L.G.; Luna,M.; Benjamin,R.S.; Keating,M.J.; Loo,T.L. Human tissue distribution of 4'-(9-acridinylamino)-methanesulfon-m-anisidide (NSC 141549, AMSA), *Cancer Chemother.Pharmacol.*, **1984**, *12*, 116–119.

Amylase

Molecular formula: indeterminate

CAS Registry No.: 9000-92-4, 9000-85-5 (bacterial), 9000-90-2 (porcine), 9000-91-3 (sweet potato)

Merck Index: 640

SAMPLE

Matrix: blood

Sample preparation: Centrifuge at 100000 g for 15 min, inject a 200 μL aliquot. Alternatively, filter (0.22 μm cellulose nitrate), inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 40 × 4 TSK SW

Column: 300 × 8 GlasPac TSK 3000 SW

Mobile phase: 10 mM pH 7.4 Phosphate buffer containing 135 mM NaCl

Flow rate: 0.8

Injection volume: 200 (titanium injector)

Detector: UV 280 or by enzyme activity

CHROMATOGRAM

Retention time: 12-16

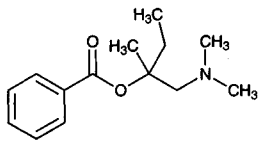
KEY WORDS

serum; GPC; SEC; human

REFERENCE

Sion,J.-P.; Laureys,M.; Gerlo,E.; Gorus,F. Detection of macroenzymes in serum by high-performance gel permeation chromatography, *J.Chromatogr.*, **1989**, *496*, 91–100.

Amylocaine



HCl

Molecular formula: $C_{14}H_{21}NO_2$
Molecular weight: 235.33
CAS Registry No.: 532-59-2, 532-59-2 (HCl)
Merck Index: 656

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben,

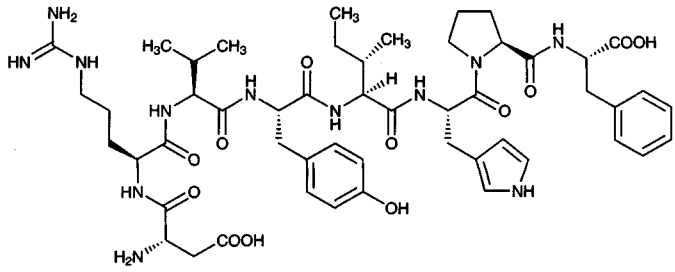
pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Angiotensin II

Molecular formula: $C_{50}H_{71}N_{13}O_{12}$
Molecular weight: 1046.19
CAS Registry No.: 4474-91-3
Merck Index: 689



SAMPLE

Matrix: blood
Sample preparation: 10 μL Serum + 240 μL 100 mM pH 7.5 potassium phosphate buffer containing 30 mM NaCl + 30 μL 1 M HCl, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-C18
Mobile phase: MeOH:0.1% trifluoroacetic acid 60:40
Column temperature: 22
Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Retention time: 5.6
Limit of quantitation: 0.16 nmole

OTHER SUBSTANCES

Extracted: angiotensin I

KEY WORDS

serum; rat

REFERENCE

Santos,R.A.S.; Krieger,E.M.; Greene,L.J. An improved fluorometric assay of rat serum and plasma converting enzyme, *Hypertension*, **1985**, *7*, 244–252.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl
Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min,

maintain at 70:30 for 1 h. Alternatively isocratic at A:B 55:45 (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)
Column temperature: 60
Flow rate: 0.3
Injection volume: 5-75
Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM
Retention time: 21 (gradient), 66 (isocratic)
Limit of detection: 100 fmole

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin, angiotensin I, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin, vasopressin

KEY WORDS
plasma; SPE; post-column reaction

REFERENCE
Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut phenyl SPE cartridge with 1 mL MeOH and 1 mL water. 6-8 mL Blood + 500 μL inhibitor solution, cool in ice, centrifuge at 4°, add 2 mL plasma to the SPE cartridge, wash with 1 mL water, elute with 500 μL MeOH then 100 μL MeOH in to coated tubes, evaporate to dryness in a vacuum centrifuge evaporator, reconstitute with 140 μL 100 mM acetic acid, centrifuge at 4° at 3000 g for 15 min, inject a 30-100 μL aliquot of the supernatant. (Inhibitor solution was EtOH:water 2:98 containing 25 mM phenanthroline, 125 mM disodium EDTA, 2 g/L neomycin, 1 mM enalaprilat, and 10 μM pepstatin. Coat polypropylene tubes by filling with 5 g/L bovine serum albumin in 100 mM pH 7.5 Tris buffer containing 200 mg/L sodium azide overnight.)

HPLC VARIABLES
Guard column: 5 μm Lichrospher RP-18 end-capped
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:86 mM pH 3.0 triethylammonium phosphate 21:79 (After 20 injections wash column with MeCN:water 80:20 for 100 min.)
Column temperature: 45
Flow rate: 1
Injection volume: 30-100
Detector: UV 210 or immunoassay

CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Simultaneous: angiotensin III

KEY WORDS
rat; SPE; plasma

REFERENCE
Huang,H.; Baussant,T.; Reade,R.; Michel,J.B.; Corvol,P. Measurement of angiotensin II concentration in rat plasma: pathophysiological applications, *Clin.Exp.Hypertens.[A].*, **1989**, *11*, 1535–1548.

SAMPLE
Matrix: blood
Sample preparation: Add enalaprilat, 1,10-phenanthroline, and tripotassium EDTA to blood to give final concentrations of 3.6 μM, 2.5 mM, and 1.5 mg/mL respectively, separate plasma by centrifuging at 4°. 2 mL Plasma + ^{125}angiotensin II, add to a hexane-washed, conditioned 500 mg Bond Elut C8 SPE cartridge, elute with two 1 mL portions of MeCN: water 50:50 containing 0.1% trifluoroacetic acid, filter the eluate, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Dynamax C8 (Rainin)
Mobile phase: Gradient. A was MeCN:water 20:80 containing 0.1% trifluoroacetic acid, adjusted to pH 4.0 with ammonium hydroxide. B was MeCN:water 35:65 containing 0.1% trifluoroacetic acid, adjusted to pH 4.0 with ammonium hydroxide. A:B from 100:0 to 0: 100 over 20 min.
Column temperature: 45
Flow rate: 1
Detector: RIA

CHROMATOGRAM
Retention time: 11.9
Internal standard: ^{125}angiotensin II (16)

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III

KEY WORDS
plasma; SPE

REFERENCE
Goldberg,M.R.; Tanaka,W.; Barchowsky,A.; Bradstreet,T.E.; McCrea,J.; Lo,M.-W.; McWilliams,E.J.,Jr.; Bjornsson,T.D. Effects of losartan on blood pressure, plasma renin activity, and angiotensin II in volunteers, *Hypertension*, **1993**, *21*, 704–713.

SAMPLE
Matrix: enzyme incubations
Sample preparation: 5 μL Serum + 70 μL 200 mM pH 7.5 phosphate buffer containing 30 mM NaCl + 20 μL 800 μM angiotensin I + 10 μL water, heat at 37° for 15 min, add 100 μL 500 mM perchloric acid, centrifuge at 800 g for 5 min. Remove a 100 μL aliquot of the supernatant and cool in ice-water, add 50 μL 5 mM benzoin in 2-methoxyethanol, add 50 μL mercaptoethanol solution, add 100 μL 0.8 M KOH, heat on a boiling water bath for 1.5 min, add 100 μL 1.2 M HCl:1 M pH 8.5 Tris-HCl buffer 50:50 (?) to adjust pH to 8.5, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water. The procedure measures the activity of angiotensin-converting enzyme in serum. The enzyme converts angiotensin I to angiotensin II which is then determined by HPLC.)

HPLC VARIABLES
Column: 250 × 4 5 μm TSK gel ODS-120T (Toyo Soda)
Mobile phase: MeOH:48 mM pH 8.5 phosphate buffer 33:67
Flow rate: 0.8
Injection volume: 100

Detector: F ex 325 em 435

CHROMATOGRAM
Retention time: 10
Limit of detection: 80-300 fmole

OTHER SUBSTANCES
Extracted: angiotensin I

KEY WORDS
derivatization

REFERENCE
Sakamoto,Y.; Miyazaki,T.; Kai,M.; Ohkura,Y. Sensitive assay for serum angiotensin-converting enzyme and separation of angiotensin analogues by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1986**, *380*, 313–320.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 mg/g solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5μm Zorbax Rx C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 15 mM pH 7.0 phosphoric acid containing 5 mM hexadecyltrimethylammonium bromide.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III

REFERENCE
Walker,T.A. Micellar HPLC: Investigation of the retention of positively charged peptides using cationic micellar mobile phases, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1715–1727.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 10 mM pH 4.15 ammonium acetate. B 1.5 mL/L acetic acid in MeOH. A:B from 80:20 to 25:75 over 45 min.
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 23.0

OTHER SUBSTANCES
Simultaneous: angiotensin I, tetradecapeptide, tetrapeptide

REFERENCE

Tonnaer,J.A.D.M.; Verhoef,J.; Wiegant,V.M.; de Jong,W. Separation and quantification of angiotensins and some related peptides by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 303–309.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 1-10 μL aliquot of an aqueous solution.

HPLC VARIABLES

Guard column: 30 × 4.6 Micro-Guard ODS-10 (Bio-Rad)
Column: 150 × 4 10 μm Bio-Sil ODS-10 (Bio Rad)
Mobile phase: MeCN:50 mM NaH_2PO_4 25:75, pH 6.0
Flow rate: 1
Injection volume: 1-10
Detector: UV 210

CHROMATOGRAM

Retention time: 3.3

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin III

REFERENCE

Guy,M.N.; Roberson,G.M.; Barnes,L.D. Analysis of angiotensins I, II, III, and iodinated derivatives by high-performance liquid chromatography, *Anal.Biochem.*, **1981**, *112*, 272–277.

SAMPLE

Matrix: solutions
Sample preparation: Cool in ice while mixing 100 μL of an aqueous solution, 50 μL 5 mM benzoin in 2-methoxyethanol, 50 μL mercaptoethanol solution, and 100 μL 0.8 M KOH, heat on a boiling water bath for 1.5 min, add 100 μL 1.6 M HCl:1 M pH 8.5 Tris-HCl buffer 50:50, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water.)

HPLC VARIABLES

Column: 15 × 4 (sic) 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 8.5 phosphate buffer 31:69
Flow rate: 0.8
Injection volume: 100
Detector: F ex 325 em 425

CHROMATOGRAM

Retention time: 5
Limit of detection: 27 fmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin III, gonadorelin, leupeptin acid, substance P, tuftsin

KEY WORDS

derivatization

REFERENCE

Kai,M.; Miyazaki,T.; Sakamoto,Y.; Ohkura,Y. Use of benzoin as pre-column fluorescence derivatization reagent for the high-performance liquid chromatography of angiotensins, *J.Chromatogr.*, **1985**, *322*, 473–477.

SAMPLE

Matrix: solutions

Sample preparation: 200 μL Solution + 100 μL chloroform + 50 μL 3 M KOH, heat at 60° for 10 min, cool in ice-water for 1 min, add 50 μL 14 M acetic acid, add 300 μL freshly prepared 265 μg/mL 1,2-diamino-4,5-dimethoxybenzene monohydrochloride in water (with cooling in ice-water), heat at 60° for 18 min, cool, inject a 100 μL aliquot. (Prepare 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39).)

HPLC VARIABLES

Column: 150 × 4 5 μm LiChrosorb RP-18

Mobile phase: MeCN:buffer:50 mM sodium 1-hexanesulfonate 26:64:10 (Prepare buffer by dissolving 14.9 g KCl in 950 mL water, adjusting pH to 2.2 with concentrated HCl, and making up to 1 L with water.)

Flow rate: 0.8

Injection volume: 100

Detector: F ex 350 em 425

CHROMATOGRAM

Retention time: 14.5

Limit of detection: 11.3 pmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin III, leucine enkephalin, methionine enkephalin

KEY WORDS

derivatization; specific for tyrosine-containing peptides

REFERENCE

Ishida,J.; Kai,M.; Ohkura,Y. High-performance liquid chromatography of tyrosine-containing peptides by pre-column derivatization involving formylation followed by fluorescence reaction with 1,2-diamino-4,5-dimethoxybenzene, *J.Chromatogr.*, **1986**, *356*, 171–177.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10-50 μL aliquot.

HPLC VARIABLES

Column: 200 × 4 5 μm TSKgel ODS-120T (Toyo Soda)

Mobile phase: Gradient. A was MeCN:200 mM pH 2.3 sodium phosphate buffer 5:95. B was MeCN:200 mM pH 2.3 sodium phosphate buffer 40:60. A:B 100:0 for 5 min, to 68.6:31.4 step gradient), to 35.7:64.3 over 45 min.

Flow rate: 1

Injection volume: 10-50

Detector: F ex 325 em 435 following post-column reaction. The column effluent mixed with reagent A pumped at 1 mL/min and the mixture flowed through a 15 m × 0.3 mm ID

PTFE coil at 76°. The effluent from this coil mixed with reagent B pumped at 0.4 mL/min and this mixture passed through a 10 × 4 column packed with 40 mg glass wool. (Prepare reagent A by mixing equal volumes of 6 mM benzoin in 2-methoxyethanol, 4.8 M KOH, and 2.1 M β-mercaptoethanol. Prepare reagent B by mixing equal volumes of 1 M Tris and 4.2 M HCl.)

CHROMATOGRAM
Retention time: 20
Limit of detection: 5-15 pmole

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III, kallidin, kyotorphin, β-melanocyte stimulating hormone, substance P
Noninterfering: estriol, estrone-3-sulfate, methionine enkephalin, phenylalanine, phenylpyruvic acid, propionic acid, sorbic acid, tryptophan, tyrosine

KEY WORDS
post-column reaction

REFERENCE
Ohno,M.; Kai,M.; Ohkura,Y. On-line post-column fluorescence derivatization of arginine-containing peptides in high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *392*, 309–316.

SAMPLE
Matrix: solutions
Sample preparation: Dry a 5 μL aliquot of a 0.1-4 mM aqueous solution, reconstitute with 100 μL pH 9.5 100 mM lithium carbonate/sodium bicarbonate buffer, add 100 μL 15 mM 9-fluorenylmethyl chloroformate in acetone, let stand at room temperature for 30 s, wash with five 500 μL portions of n-pentane. Remove a 10 μL aliquot of the aqueous phase and add it to 90 μL 10 mM NaH_2PO_4, mix, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 125 × 2 5 μm LiChrospher RP-2
Mobile phase: MeCN:10 mM pH 4.9 NaH_2PO_4 54:46
Flow rate: 1
Injection volume: 2
Detector: F ex 260 em 310

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 500 fmole

KEY WORDS
derivatization

REFERENCE
Vogt,W.; Egeler,E.; Sommer,W.; Eisenbeiss,F.; Meyer,H.D. High-performance liquid chromatographic determination of hormonal peptides and their fluorenylmethoxycarbonyl derivatives, *J.Chromatogr.*, **1987**, *400*, 83–89.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 20 μL aliquot with 20 μL 500 mM pH 9.0 potassium phosphate buffer and 30 μL 2 mg/mL fluorescamine, mix, let stand at room temperature for 5 min, inject a 10-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 5 μm Ultrasphere octylsilica

Mobile phase: Gradient. MeOH:buffer from 40:60 to 65:35 over 10 min, maintain at 65:35 for 10 min. (Prepare buffer by dissolving 1.21 g Tris and 2.8 mL triethylamine in 1 L water, adjust pH to 7.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 0.3
Injection volume: 10-50
Detector: F ex 390 em 470 (cutoff filter)

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III

KEY WORDS
derivatization

REFERENCE
Boppana,V.K.; Miller-Stein,C.; Politowski,J.F.; Rhodes,G.R. High-performance liquid chromatographic determination of peptides in biological fluids by automated pre-column fluorescence derivatization with fluorescamine, *J.Chromatogr.*, **1991**, *548*, 319–327.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of a 25 μM peptide solution in water with 50 μL 200 mM ascorbic acid in water, 100 μL 10 mM NaCN in water, 200 μL 5 mM naphthalene-2,3-dicarboxaldehyde in MeCN, and 540 μL 100 mM pH 7.0 phosphate buffer, mix, let stand at 0-4° for 20 min, add 50 μL 200 mM taurine in water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil
Mobile phase: THF:100 mM pH 6.0 phosphate buffer 19:81 containing 30 mM sodium 1-octanesulfonate
Column temperature: 40 ± 0.1
Flow rate: 1-2
Detector: F ex 420 em 490

CHROMATOGRAM
Retention time: k' 11
Limit of detection: 50-100 fmole

OTHER SUBSTANCES
Simultaneous: similar peptides

KEY WORDS
derivatization

REFERENCE
Patel,H.B.; Stobaugh,J.F.; Riley,C.M. Reversed-phase ion-pair liquid chromatography of the angiotensins, *J.Chromatogr.*, **1991**, *536*, 357–370.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 5 μm Kromasil C8 (Eka-Nobel)

Mobile phase: Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was MeCN:water 90:10 containing 0.1% trifluoroacetic acid. A:B from 0:100 to 75:25 over 8 min, to 25:75 over 12 min.
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7

OTHER SUBSTANCES
Simultaneous: angiotensin I, bradykinin, insulin, leucin enkephalin, lysozyme, melittin, methionine enkephalin, oxytocin

REFERENCE
Supelco Catalog, **1992,** p. 104.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 4.6 5 μm Hypersil WP 300-octyl
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in n-propanol. A:B 100:0 for 2.5 min, to 90:10 over 2.5 min, to 10:90 over 112 min.
Flow rate: 1
Detector: UV 225

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: bombesin, bovine growth hormone, bovine serum albumin, catalase, lysozyme, L-tryptophan

REFERENCE
Supelco Catalog, **1993,** p. 602.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: angiotensin I, calcitonin (human), α-endorphin, β-endorphin, gonadorelin (LH-RH), protirelin (TRH), somatostatin

REFERENCE
Varian Catalog, **1993,** p. 182.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Vydac 228TP
Mobile phase: Gradient. A was 0.25% trifluoroacetic acid in water. B was 0.25% trifluoroacetic acid in MeCN:water 70:30. A:B from 95:5 to 0:100 over 30 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: angiotensin I, bradykinin, eledosin, insulin, lysozyme, myoglobin, neurotensin, ovalbumin, oxytocin

REFERENCE
Supelco Catalog, **1993,** p. 581.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mM solution in 100 mM pH 6 morpholinoethanesulfonic acid/NaOH buffer. Remove a 20 μL aliquot and add it to 5 μL 25 mM 5-carboxytetramethylrhodamine succinimidyl ester (Molecular Probes, Eugene OR) in DMF, let stand at room temperature overnight, add 50 μL 1 M pH 8 Tris-HCl buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: reversed-phase
Column: reversed-phase
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 55:45 "applied just after application of a sample".
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 38

OTHER SUBSTANCES
Simultaneous: angiotensin I

KEY WORDS
derivatization

REFERENCE
Shimura,K.; Kasai,K.-I. Fluorescence-labeled peptides as isoelectric point (p*I*) markers in capillary isoelectric focusing with fluorescence detection, *Electrophoresis*, **1995**, *16*, 1479–1484.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax 300 Å SB-C3

Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 5:95:0.1. B was MeCN:water:trifluoroacetic acid 5:95:0.085. A:B from 85:15 to 47:53 over 20 min.
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: carbonic anhydrase, cytochrome C, insulin, leucine enkephalin, lysozyme, myoglobin, RNAase

REFERENCE
Ricker,R.D.; Sandoval,L.A.; Permar,B.J.; Boyes,B.E. Improved reversed-phase high performance liquid chromatography columns for biopharmaceutical analysis, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 93–105.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH, 10 mL THF, 10 mL hexane, 10 mL MeOH, and 10 mL water. Homogenize (Polytron) tissue with 20 volumes of ice-cold EtOH:180 mM HCl 75:25, centrifuge at 4° at 32570 g for 20 min, store the supernatant at -20° for 18-20 h, centrifuge at 4° at 2200 g for 20 min. Adjust the pH of the supernatant to 6.5 with 1 M NaOH, store at 4° for 1 h, centrifuge at 4° at 2200 g for 20 min, evaporate to dryness under reduced pressure, reconstitute with 10 mL pH 3 0.1% trifluoroacetic acid, add to the SPE cartridge, wash with water, wash with 10 mL MeCN:0.1% trifluoroacetic acid 10:90, elute with 10 mL MeCN:0.1% trifluoroacetic acid 30:70, inject a 50-200 μL aliquot of the eluate.

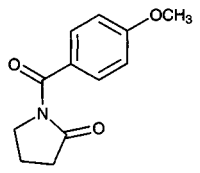
HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: Gradient. A was MeCN:25 mM pH 7.6 phosphate buffer 5:95. B was MeCN:25 mM pH 7.6 phosphate buffer 95:5. A:B from 89:11 to 68:32 over 12 min (Waters curve 7).
Flow rate: 1.5
Injection volume: 50-200
Detector: UV 214 or radioimmunoassay

CHROMATOGRAM
Retention time: 8

KEY WORDS
rat; adrenal; brain; heart; kidney; liver; lung; ovary; plasma; testes; uterus; SPE

REFERENCE
De Silva,P.E.; Husain,A.; Smeby,R.R.; Khairallah,P.A. Measurement of immunoreactive angiotensin peptides in rat tissues: some pitfalls in angiotensin II analysis, *Anal.Biochem.*, **1988**, *174*, 80–87.

Aniracetam



Molecular formula: $C_{12}H_{13}NO_3$
Molecular weight: 219.24
CAS Registry No.: 72432-10-1
Merck Index: 700

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma, 100 μL 50 μ g/mL IS in MeCN, mix, centrifuge at 12000 rpm for 10 min. Filter (0.45 μ m) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Inertsil ODS
Mobile phase: MeCN:water:acetic acid 25:75:0.5
Flow rate: 1.0
Detector: UV 254

CHROMATOGRAM
Internal standard: aspirin
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Ogiso,T.; Iwaki,M.; Tanino,T.; Ikeda,K.; Paku,T.; Horibe,Y.; Suzuki,H. Pharmacokinetics of aniracetam and its metabolites in rats, *J.Pharm.Sci.*, **1998**, *87*, 594–598.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 25 μL 5 μg/mL IS in water, mix thoroughly. Maintain sample at 4° until injection, inject 200 μL plasma onto column A with mobile phase A, after 4 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, monitor the effluent from column B. Wash column A with MeOH for 10.8 min, re-equilibrate with water for 10 min. At the end of the separation backflush column A with mobile phase B for 12 min.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS; B 30 × 4.6 10 μm Brownlee RP-2 + 60 × 4 3 μm Hypersil ODS
Mobile phase: A water; B MeOH:MeCN:water 30:10:70
Flow rate: A 2; B 1
Injection volume: 200
Detector: UV 282

CHROMATOGRAM
Retention time: 8.3
Internal standard: 1-(p-ethoxybenzoyl)-2-pyrrolidinone (Ro 13-8606) (11.8)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; column switching

REFERENCE

Guenzi,A.; Zanetti,M. Determination of aniracetam and its main metabolite, N-anisoyl-GABA, in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *530*, 397–406.

Anistreplase

Molecular formula: indeterminate
CAS Registry No.: 81669-57-0
Merck Index: 712

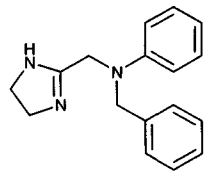
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Bakerbond C4
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 60:40 over 90 min
Flow rate: 1
Injection volume: 200
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: alteplase, streptokinase, urokinase

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

Antazoline



Molecular formula: $C_{17}H_{19}N_3$
Molecular weight: 265.36
CAS Registry No.: 91-75-8, 2508-72-7
Merck Index: 718
Lednicer No.: 1 242

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 11.04

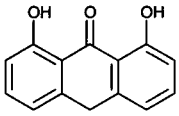
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Anthralin



Molecular formula: $C_{14}H_{10}O_3$
Molecular weight: 226.23
CAS Registry No.: 1143-38-0
Merck Index: 723

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in dichloromethane. Mix a 1 mL aliquot with 19 mL dichloromethane and 1 mL acetic acid, make up to 50 mL with hexane. Inject an aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP18
Column: 250 × 4 5 μm LiChrospher 100 RP18
Mobile phase: MeOH:water:acetic acid 77:23:0.1, adjusted to pH 5.5 with conc. ammonia
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 15.6

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Müller,K.; Ziereis,K.; Wiegrebe,W. The monograph dithranol in the European Pharmacopoeia -comment and amendments, *Pharmazie*, **1996**, *51*, 980–981.

SAMPLE

Matrix: formulations
Sample preparation: Creams, gels, lotions. Disperse and dilute in MeCN to an anthralin concentration of 0.25 mg/mL. Remove a 10 mL aliquot and add it to 10 mL 1 mg/mL anthracene in MeCN, 10 mL chloroform, 20 mL MeCN, and 0.25 mL acetic acid. Filter (Whatman No. 42 paper), inject a 10 μL aliquot. Sticks, ointments. Disperse and dilute in chloroform to an anthralin concentration of 0.25 mg/mL. Remove a 10 mL aliquot and add it to 10 mL 1 mg/mL anthracene in MeCN, 30 mL MeCN, and 0.25 mL acetic acid. Filter (Whatman No. 42 paper), inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 60:39.5:0.5 containing 0.05% sodium hexanesulfonate
Flow rate: 2.5
Injection volume: 10
Detector: UV 365

CHROMATOGRAM

Retention time: 6.5
Internal standard: anthracene (8.5)

OTHER SUBSTANCES

Simultaneous: danthron, dianthrone

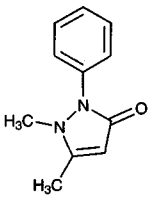
KEY WORDS

protect from light; deaerate all solvents with argon; creams; gels; lotions; sticks; ointments

REFERENCE

Burton,F.W.; Gadde,R.R. Analysis of anthralin in dermatological products by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *328*, 317–324.

Antipyrine



Molecular formula: $C_{11}H_{12}N_2O$
Molecular weight: 188.23
CAS Registry No.: 60-80-0, 520-07-0 (salicylate)
Merck Index: 757
Lednicer No.: 1 234

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. 50 μL Plasma or amniotic fluid + 50 μL 5 μg/mL 3-hydroxyacetamidophenol in water, add to SPE cartridge, wash twice with 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate under vacuum, reconstitute the residue in 100 μL MeCN:water 6:94, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: Gradient. A was MeCN:pH 4.0 ammonium phosphate buffer 6:94. B was MeCN:pH 4.0 ammonium phosphate buffer 25:75. A:B from 100:0 to 100:0 over 20 min, to 100:0 over 5 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES

Extracted: didanosine

KEY WORDS

plasma; monkey; pharmacokinetics; SPE

REFERENCE

Pereira,C.M.; Nosbisch,C.; Winter,H.R.; Baughman,W.L.; Unadkat,J.D. Transplacental pharmacokinetics of dideoxyinosine in pigtailed macaques, *Antimicrob.Agents Chemother.*, **1994**, *38*, 781–786.

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. 100 μL Plasma or amniotic fluid + 20 μL 10 μg/mL 3-hydroxyacetamidophenol in water, mix, add to the SPE cartridge, wash with two 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute with 100 μL MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94. B was MeCN:50 mM pH 3.3 ammonium phosphate buffer 25:75. A:B from 100:0 to 0:100 over 17 min, maintain at 0:100 for 5 min, return to initial conditions over 3 min, re-equilibrate for 17 min.
Flow rate: 1
Injection volume: 50

Detector: UV 266

CHROMATOGRAM
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: stavudine

KEY WORDS
monkey; plasma; pharmacokinetics; SPE

REFERENCE
Odinecs,A.; Nosbisch,C.; Keller,R.D.; Baughman,W.L.; Unadkant,J.D. In vivo maternal-fetal pharmacokinetics of stavudine (2',3'-didehydro-3'-deoxythymidine in pigtailed macaques (*Macaca nemestrina*), *Antimicrob.Agents Chemother.*, **1996**, *40*, 196–202.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 0.5 mL water + 0.5 mL 0.25 M NaOH, vortex, allow to stand at room temperature for 20 min, add 20 μL of 100 μg/mL phenacetin and 3 μg/mL flunitrazepam, vortex, add 5 mL diethyl ether, vortex for 30 s, centrifuge at 900 g for 5 min, freeze in acetone/dry ice for 5 min. Remove the supernatant and dry it under nitrogen. Reconstitute in 115 μL MeCN:0.1% pH 3 sodium phosphate buffer 30:70, inject an aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 37-50 μm μBondapak phenyl
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. A was MeCN:0.1% pH 3 sodium phosphate buffer 5:95. B was MeCN:0.1% pH 3 sodium phosphate buffer 70:30. A:B 80:20 for 2.5 min, then to 45:55 over 20 min, then to 25:75 over 3 min, then to 80:20 over 3 min, equilibrate at 80:20 for 7 min.
Column temperature: 40
Flow rate: 2
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 5.08
Internal standard: phenacetin (7.09)
Limit of quantitation: 5500 ng/mL

OTHER SUBSTANCES
Simultaneous: lorazepam (at 229 nm)
Noninterfering: acetaminophen, trimethoprim, sulfamethoxazole, allopurinol, indocyanine green

KEY WORDS
plasma

REFERENCE
Riley,C.A.; Evans,W.E. Simultaneous analysis of antipyrine and lorazepam by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *382*, 199–205.

SAMPLE
Matrix: blood

Sample preparation: 0.5 mL Plasma +10 μL 250 μg/mL 1-acetamidopyrene in MeOH + 200 μL 1 M ammonium sulfate + 800 μL cold MeCN, vortex for 30 s, store at -20° for at least 30 min, vortex, centrifuge at 1500 g for 30 min. Remove 400 μL of the upper organic layer and evaporate it under a stream of nitrogen. Reconstitute with 100 μL mobile phase, vortex for 30 s, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 47:53 (Buffer was 6.805 g potassium monophosphate in 1 L water, adjust pH to 6.00 with 10 M NaOH.)
Flow rate: 1
Injection volume: 75
Detector: UV 214

CHROMATOGRAM

Retention time: 4.2
Internal standard: 1-acetamidopyrene (9.7)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: lorazepam, indocyanine green
Noninterfering: adenosine, albuterol, alphenal, aspirin, caffeine, carbamazepine, cefazolin, cephalexin, cephalothin, cimetidine, ciprofloxacin, claforan, desipramine, enoxacin, fleroxacin, furosemide, hydralazine, hydrochlorothiazide, minoxidil, norfloxacin, phenytoin, propafenone, sulindac, teicoplanin, theophylline, vancomycin
Interfering: some indocyanine green impurities

KEY WORDS

plasma

REFERENCE

Awni,W.M.; Bakker,L.J. Antipyrine, indocyanine green, and lorazepam determined in plasma by high-pressure liquid chromatography, *Clin.Chem.*, **1989**, *35*, 2124–2126.

SAMPLE

Matrix: blood
Sample preparation: Make plasma alkaline with NaOH, extract with dichloromethane.

HPLC VARIABLES

Column: reverse phase
Mobile phase: MeOH:water 43:57 containing 100 mM triethylamine, adjusted to pH 4.7 with orthophosphoric acid
Detector: UV 254

CHROMATOGRAM

Internal standard: phenacetin
Limit of detection: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Hayton,W.L.; Kneer,J.; Blouin,R.A.; Stoeckel,K. Pharmacokinetics of intravenous cefetamet and oral cefetamet pivoxil in patients with hepatic cirrhosis, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1318–1322.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Serum + 200 μL mobile phase, filter (Millipore Millex 0.45 μm), inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 C18 Corasil II
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Propanol:6 mM C_{12} DAPS (Fluka) 3:97 (C_{12} DAPS is 3-(dimethyldodecylammonio) propanesulfonate.)
Injection volume: 25
Detector: UV 273

CHROMATOGRAM
Retention time: 8
Internal standard: antipyrine

OTHER SUBSTANCES
Simultaneous: theophylline, β-hydroxytheophylline, caffeine, theobromine, albendazole, albendazole sulfoxide, nimorazole, flubendazole, mercaptopurine, aminophylline, amyleine, procaine
Interfering: metronidazole, tinidazole, dipropyline

KEY WORDS
serum; micellar chromatography; antipyrine is IS

REFERENCE
Habel,D.; Guermouche,S.; Guermouche,M.H. Direct determination of theophylline in human serum by high-performance liquid chromatography using zwitterionic micellar mobile phase. Comparison with an enzyme multiplied immunoassay technique, *Analyst*, **1993**, *118*, 1511–1513.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 1 μg phenacetin + 100 μL 100 mM NaOH + 400 μL water + 3 mL dichloromethane, shake vigorously for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:10 mM pH 8 phosphate buffer 20:80
Flow rate: 1.5
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: phenacetin (12)
Limit of detection: <500 ng/mL

KEY WORDS
plasma

REFERENCE
Wolfisberg,H.; Schmutz,A.; Stotzer,R.; Thormann,W. Assessment of automated capillary electrophoresis for therapeutic and diagnostic drug monitoring: determination of bupivacaine in drain fluid and antipyrine in plasma, *J.Chromatogr.A*, **1993**, *652*, 407–416.

SAMPLE
Matrix: blood

Sample preparation: 50 μL Plasma + 500 μL 370 mM pH 4.6 acetate buffer + cyclobarbital + 5 mL dichloromethane, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 70 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Cyclobond II (Baker)
Column: 250 × 4.6 Cyclobond II (Baker)
Mobile phase: MeOH:water 15:85
Flow rate: 0.7
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Internal standard: cyclobarbital
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: hexobarbital, phenobarbital

KEY WORDS

plasma; rat

REFERENCE

Groen,K.; Breimer,D.D.; Jansen,E.J.; Van Bezooijen,C.F.A. The influence of aging on the metabolism of simultaneously administered hexobarbital enantiomers and antipyrine before and after phenobarbital induction in male rats: A longitudinal study, *J.Pharmacol.Exp.Ther.*, **1994**, *268*, 531–536.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL cefadroxil + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:0.5 mM phosphoric acid 12:88
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Internal standard: cefadroxil

KEY WORDS

plasma; rat

REFERENCE

Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 40 μg/mL 3-acetamidophenol extracted with ether:dichloromethane:isopropanol 60:40:1. Evaporate organic layer under a stream of nitrogen and take up residue in 250 μL 50 mM pH 7.8 phosphate buffer.

HPLC VARIABLES
Column: 100 × 4.6 Chrompack microsphere C18
Mobile phase: Gradient. A was 50 mM pH 7.8 buffer. B was MeOH:50 mM pH 7.8 phosphate buffer 50:50. A:B from 100:0 to 50:50 over 38 min, maintain at 50:50 for 2 min.
Flow rate: 0.7
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Internal standard: 3-acetamidophenol

OTHER SUBSTANCES
Simultaneous: acetaminophen, metabolites

KEY WORDS
plasma; pig

REFERENCE
Monshouwer,M.; Witkamp,R.F.; Pijpers,A.; Verheijden,J.H.M.; Van Miert,A.S.J.P.A.M. Dose-dependent pharmacokinetic interaction between antipyrine and paracetamol *in vivo* and *in vitro* when administered as cocktail in pig, *Xenobiotica*, **1994**, *24*, 347–355.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 40 μg/mL 3-acetamidophenol extracted with ether:dichloromethane:isopropanol 60:40:1. Evaporate organic layer under a stream of nitrogen and take up residue in 50 mM pH 7.8 phosphate buffer, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Chrompack microsphere C18
Mobile phase: Gradient. A was 50 mM pH 7.8 phosphate buffer. B was MeOH:50 mM pH 7.8 phosphate buffer 50:50. A:B from 100:0 to 50:50 over 38 min, maintain at 50:50 for 2 min.
Flow rate: 0.7
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Internal standard: 3-acetamidophenol

OTHER SUBSTANCES
Extracted: acetaminophen

KEY WORDS
plasma; metabolites; pig; pharmacokinetics

REFERENCE
Monshouwer,M.; Witkamp,R.F.; Pijpers,A.; Verheijden,J.H.M.; Van Miert,A.S.J.P.A.M. Dose-dependent pharmacokinetic interaction between antipyrine and paracetamol *in vivo* and *in vitro* when administered as cocktail in pig, *Xenobiotica*, **1994**, *24*, 347–355.

SAMPLE
Matrix: blood
Sample preparation: Plasma + phenacetin + 10% trichloroacetic acid, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Nucleosil C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 50 mM citrate adjusted to pH 5 with acetic acid.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 20
Detector: UV 244

CHROMATOGRAM

Internal standard: phenacetin
Limit of quantitation: 500 ng/mL

KEY WORDS

dog; plasma; pharmacokinetics

REFERENCE

Galtier,M.; Briand,D.; Pinguet,F.; Gomeni,R.; Fabre,D.; Bressolle,F. Pharmacokinetic parameters of antipyrine in dog after hepatectomy, *Biopharm.Drug Dispos.*, **1995**, *16*, 669–684.

SAMPLE

Matrix: blood, saliva
Sample preparation: 1 mL Plasma or saliva + 100 μL 400 μg/mL phenacetin in EtOH + 100 μL 2 M NaOH + 5 mL dichloromethane:n-pentane 50:50, vortex for 15 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 10 μm Spheri-10 RP-18
Mobile phase: MeCN:100 mM sodium acetate:triethylamine 7.5:92:0.5, pH 6.6
Column temperature: 40
Flow rate: 3.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Internal standard: phenacetin

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Jorquera,F.; Almar,M.M.; Jimeno,A.; González-Sastre,M.; González-Gallego,J. Assessment of antipyrine kinetics from saliva or plasma: influence of age, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1141–1145.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Mix 500 μL plasma with 25 μL 15 μg/mL IS in MeOH, 500 μL 750 mM pH 5.0 sodium acetate buffer containing 40 mg/mL sodium metabisulfite, and 10 mg of a mixture of β-glucuronidase and arylsulfatase (Limpet acetone powder Type I: Platela vulgata, Sigma). Heat at 37° for 2 h, add 100 mg NaCl, extract with 1.2 mL chloroform:EtOH 90:10 for 2 min (Caution! Chloroform is a carcinogen!). Centrifuge at 1800 g for 20 min, add the organic phase to 50 μL 100 mM HCl, evaporate to dryness under a stream of nitrogen, dissolve the residue in 75 μL mobile phase, wash with 25 μL n-hexane, inject a 20 μL aliquot of the lower phase. Urine. Mix 500 μL urine with 25 μL 500 μg/mL IS in MeOH, 500 μL 750 mM pH 5.0 sodium acetate buffer containing 40 mg/mL sodium metabisulfite, and 10 mg of a mixture of β-glucuronidase and arylsulfatase

(Limpet acetone powder Type I: Platela vulgata, Sigma). Heat at 37° for 2 h, add 200 mg sodium chloride, extract with 2 mL dichloromethane:isopropanol 90:10 for 90 s, centrifuge at 1800 g for 30 min, evaporate the organic phase under a stream of nitrogen. Dissolve the residue in 200 μL mobile phase, vortex for 15 s, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:250 mM pH 5.0 sodium acetate buffer 30:70
Flow rate: 1.0
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: phenacetin (10)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lanchote,V.L.; Ping,W.C.; Santos,S.R.C.J. Determination of antipyrine and metabolites in plasma of a patient with mild renal failure, *Ther.Drug Monit.*, **1997**, *19*, 705–710.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 500 μL 100 mM NaOH + 10 mL benzene (CAUTION! Benzene is a carcinogen!), shake vigorously for 20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 20:75:5
Flow rate: 1
Injection volume: 25
Detector: UV 254 for 7 min then UV 280

CHROMATOGRAM
Retention time: 8.0
Internal standard: antipyrine

OTHER SUBSTANCES
Extracted: 4-monomethylaminoantipyrine

KEY WORDS
plasma; antipyrine is IS

REFERENCE
Asmardi,G.; Jamali,F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids, *J.Chromatogr.*, **1983**, *277*, 183–189.

SAMPLE
Matrix: saliva
Sample preparation: Add 100 μL phenacetin to 1 mL saliva, inject an aliquot.

HPLC VARIABLES
Column: 10 μm Spheri-10 RP-18 (Brownlee)
Mobile phase: MeCN:buffer 7.5:92.5 (The buffer was 100 mM sodium acetate containing 0.5% triethylamine, pH 6.6.)
Column temperature: 40
Flow rate: 3.5
Detector: UV 254

CHROMATOGRAM
Internal standard: phenacetin

KEY WORDS
pharmacokinetics; saliva

REFERENCE
Jorquera,F.; Almar,M.M.; Gonzalez-Sastre,M.; Suarez,I.; Gonzalez-Gallego,J. Accuracy of the one-sample method for determination of antipyrine clearance in elderly subjects, *J.Pharm.Biomed.Anal.*, **1996**, *15*, 7–11.

SAMPLE
Matrix: saliva
Sample preparation: 500 μL Saliva + 500 μL MeCN + 50 μL 400 μg/mL phenacetin, vortex, centrifuge at 5500 g for 1 min. Filter (4.5 μm) the supernatant and inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Eicopack MA-ODS (Eicom, Kyoto)
Mobile phase: MeCN:20 mM pH 6.0 potassium phosphate buffer 30:70 containing 0.1% triethylamine
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: phenacetin (8.2)
Limit of detection: 100 ng/mL

REFERENCE
Echizen,H.; Nakura,M.; Ishizaki,I. Rapid and simple high-performance liquid chromatographic determination of saliva antipyrine for routine antipyrine test, *J.Chromatogr.*, **1990**, *526*, 296–299.

SAMPLE
Matrix: saliva
Sample preparation: 1 mL Saliva + 100 μL 2 M NaOH + 100 μL 400 μg/mL phenacetin in EtOH + 5 mL n-pentane:dichloromethane 50:50, extract on a whirlmixer for 15 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 10 μm Spheri-10 RP-18
Mobile phase: MeCN:100 mM sodium acetate:triethylamine 7.5:92:0.5, pH 6.6
Column temperature: 40
Flow rate: 3.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Internal standard: phenacetin

KEY WORDS
pharmacokinetics

REFERENCE
Jorquera,F.; Almar,M.M.; Pozuelo,M.; Sansegundo,D.; González-Sastre,M.; González-Gallego,J. Effects of aging on antipyrine clearance: Predictive factors of metabolizing capacity, *J.Clin.Pharmacol.*, **1995**, *35*, 895–901.

SAMPLE
Matrix: saliva
Sample preparation: Saliva + phenacetin + MeCN, vortex, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS-Technosphere (HPLC Technology)
Mobile phase: MeCN:50 mM pH 6.0 sodium phosphate buffer 1:2
Flow rate: 1.5
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Internal standard: phenacetin
Limit of detection: 100 nM

REFERENCE
Perrett,D.; Ross,G.A. Rapid determination of drugs in biofluids by capillary electrophoresis. Measurement of antipyrine in saliva for pharmacokinetic studies, *J.Chromatogr.A*, **1995**, *700*, 179–186.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 400 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: chlorpheniramine

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.60

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazo-

cine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.39 (A), 4.54 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone,

phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 150 × 3 4 μm Supersphere 100 RP18 endcapped (Bischoff)
Mobile phase: MeCN:20 mM pH 7.4 KH_2PO_4 10:90
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: degradation products, radiation degradation products, o-hydroxyantipyrine, m-hydroxyantipyrine, p-hydroxyantipyrine

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Coolen,S.A.J.; Everaerts,F.M.; Huf,F.A. Characterization of ^{60}Co γ-radiation induced radical products of antipyrineby means of high-performance liquid chromatography, mass spectrometry, capillary zone electrophoresis, micellar electrokinetic capillary chromatography and nuclear magnetic resonance spectrometry, *J.Chromatogr.A*, **1997**, *788*, 95–103.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.70

OTHER SUBSTANCES
Also analyzed: amoxicillin, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Condition a C18 AASP SPE cartridge (Varian) with 1 mL MeOH and 1 mL water. 100 μL Urine + 100 μL 50 mM pH 4.5 KH_2PO_4 + 20 mg limpet acetone powder (type I, contains β-glucuronidase and sulfatase, from Sigma), + 16 mg sodium metabisulfite, vortex for 15 s, heat at 40° with mild agitation for 3 h. Cool to room temperature, add 10 μL 1 mg/mL 4-dimethylaminoantipyrine in water, vortex, centrifuge at 500 g for 20 min. Remove 100 μL supernatant and add it to 900 μL pH 4.5 100 mM TRIS. Remove 500 μL and add it to 500 μL 50 mM pH 4.5 KH_2PO_4, add to SPE cartridge, wash with four 1 mL portions of 50 mM pH 4.5 phosphate buffer, allow to dry completely, elute with three 100 μL volumes of MeCN:dichloromethane 20:80. Evaporate eluate at 40° under vacuum while vortexing, reconstitute the residue in 100 μL 50 mM pH 4.5 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 40 μm Corasil phenyl
Column: 100 × 4.6 5 μm Spherisorb phenyl
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeOH. A:B from 90:10 to 60:40 over 14 min, to 40:60 over 7 min, to initial conditions over 0.1 min, re-equilibrate for 7.9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 17.8
Internal standard: 4-dimethylaminoantipyrine (19.9)
Limit of quantitation: 2000 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Sarkar,M.A.; March,C.; Karnes,H.T. Solid phase extraction and simultaneous high performance liquid chromatographic determination of antipyrine and its major metabolites in urine, *Biomed.Chromatogr.*, **1992**, *6*, 300–304.

SAMPLE
Matrix: urine
Sample preparation: Inject a 100 μL aliquot directly.

HPLC VARIABLES
Guard column: 25 × 4 5 μm LiChrospher 100 RP 18
Column: 125 × 4 5 μm LiChrospher 60 RP Select B
Mobile phase: Gradient. A was 5 mM tetrabutylammonium phosphate in water. B was 5 mM tetrabutylammonium phosphate in MeOH. A:B from 90:10 to 87:13 over 10 min, to 67:33 over 16 min, to 60:40 over 9 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, return to initial conditions over 5 min.
Flow rate: 0.7
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 23

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog

REFERENCE
Velic,I.; Metzler,M.; Hege,H.G.; Weymann,J. Separation and identification of phase I and phase II [^{14}C]antipyrine metabolites in rat and dog urine, *J.Chromatogr.B*, **1995**, *666*, 139–147.

Antitrypsin

Molecular formula: indeterminate
CAS Registry No.: 9041-92-3
Merck Index: 760

SAMPLE

Matrix: blood
Sample preparation: Dialyze 2 mL plasma against 30 mM pH 7.0 sodium phosphate buffer overnight, chromatograph on 20 mL Blue-Sepharose (equilibrated with 30 mM pH 7.0 sodium phosphate buffer) at 40 mL/h. Dialyze non-retained fraction (20-30 mL) against 30 mM pH 5.8 sodium acetate buffer overnight, chromatograph on 10 mL Red-Sepharose (equilibrated with 30 mM pH 5.8 sodium acetate buffer). Concentrate non-retained fraction (30-50 mL) to 2-3 mL with Amicon PM-10 and freeze dry, dissolve freeze-dried sample in 300 μL 10 mM pH 7.0 sodium phosphate buffer containing 100 mM NaCl, inject into HPLC.

HPLC VARIABLES

Column: 100 × 6 KB-column hydroxy-apatite (Koken)
Mobile phase: Gradient. A was 10 mM pH 7.0 sodium phosphate buffer containing 100 mM NaCl. B was 200 mM pH 7.0 sodium phosphate buffer containing 100 mM NaCl. A:B from 100:0 to 50:50 over 1 h.
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 22.3

OTHER SUBSTANCES

Extracted: α1-acid glycoprotein

KEY WORDS

plasma

REFERENCE

Funae,Y.; Wada,S.; Imaoka,S.; Hirotsune,S.; Tominaga,M.; Tanaka,S.; Kishimoto,T.; Maekawa,M. Chromatographic separation of *α1*-acid glycoprotein from *α1*-antitrypsin by high-performance liquid chromatography using a hydroxyapatite column, *J.Chromatogr.*, **1986**, *381*, 149–152.

SAMPLE

Matrix: blood
Sample preparation: Prepare concentrate from plasma (procedure given).

HPLC VARIABLES

Column: superose-12 (Pharmacia)
Mobile phase: 500 mM NaCl
Flow rate: 0.5
Injection volume: 50
Detector: UV 280

KEY WORDS

plasma

REFERENCE

Burnouf,T.; Constans,J.; Clerc,A.; Descamps,J.; Martinache,L.; Goudemand,M. Biochemical and biological properties of an α_1-antitrypsin concentrate, *Vox Sang.*, **1987**, *52*, 291–297.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in PBS.

HPLC VARIABLES

Column: 600 × 7.5 Spheragel-TSK 3000 SW
Mobile phase: PBS
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 16.4

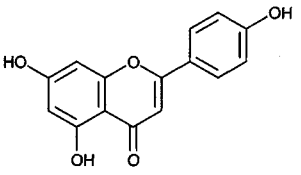
OTHER SUBSTANCES

Simultaneous: aggregates

REFERENCE

Glaser,C.B.; Busby,T.F.; Ingham,K.C.; Childs,A. Thermal denaturation of α 1-protease inhibitor. Stabilization by neutral salts and sugars, *Am.Rev.Respir.Dis.*, **1983**, *128*, 77–81.

Apigenin



Molecular formula: $C_{15}H_{10}O_5$
Molecular weight: 270.24
CAS Registry No.: 520-36-5
Merck Index: 773

SAMPLE

Matrix: tissue
Sample preparation: Homogenize epidermal cells in absolute EtOH. Evaporate the solvent, dissolve the residue in 100 μL absolute EtOH containing 2.4 μg/mL IS. Filter (0.20 μm nylon membrane), inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 Alltima C18 (Alltech)
Mobile phase: MeCN:water 48:52 containing 0.1% trifluoroacetic acid
Flow rate: 0.3
Injection volume: 5
Detector: UV 337

CHROMATOGRAM

Retention time: 3.76
Internal standard: quercetin (3.06)
Limit of detection: 1.15 ng

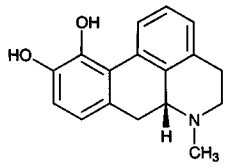
KEY WORDS

epidermal cells; mouse

REFERENCE

Li,B.; Robinson,D.H.; Birt,D.F. Evaluation of properties of apigenin and [G-3H]apigenin and analytic method development, *J.Pharm.Sci.*, **1997**, *86*, 721–725.

Apomorphine



Molecular formula: $C_{17}H_{17}NO_2$
Molecular weight: 267.33
CAS Registry No.: 58-00-4, 41372-20-7 (HCl)
Merck Index: 787

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 8.962

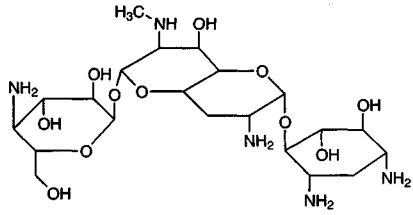
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Apramycin



Molecular formula: $C_{21}H_{41}N_5O_{11}$
Molecular weight: 539.58
CAS Registry No.: 37321-09-8
Merck Index: 792

SAMPLE

Matrix: fermentation solutions
Sample preparation: 5 mL Fermentation broth + 5 mL saturated aqueous solution of Tris + 20 mL MeCN, centrifuge at 3000 rpm for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 3 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° under a reflux condenser for 45 min, make up to 4 mL with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:water:acetic acid 55:45:0.15
Flow rate: 1.2
Injection volume: 20
Detector: UV 350

CHROMATOGRAM

Retention time: 9.56

OTHER SUBSTANCES

Extracted: kanamycin B, tobramycin

KEY WORDS

derivatization

REFERENCE

Harangi,J.; Deák,M.; Nánási,P.; Bacsa,G. Determination of the major factors of fermentation of the nebramycin complex by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 83–93.

SAMPLE

Matrix: reaction mixtures
Sample preparation: 50 μL Buffered reaction mixture + 50 μL isopropanol + 50 μL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 μL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

HPLC VARIABLES

Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 70:30.
Flow rate: 2
Injection volume: 50
Detector: UV 330

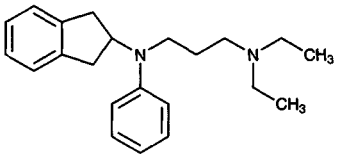
CHROMATOGRAM

Retention time: 20.5

REFERENCE

Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

Aprindine



Molecular formula: $C_{22}H_{30}N_2$
Molecular weight: 322.49
CAS Registry No.: 37640-71-4, 33237-74-0 (HCl)
Merck Index: 793

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.967

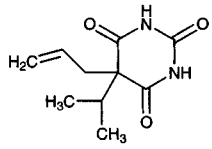
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Aprobarbital



Molecular formula: $C_{10}H_{14}N_2O_3$
Molecular weight: 210.23
CAS Registry No.: 77-02-1
Merck Index: 794
Lednicer No.: 1 268

SAMPLE

Matrix: blood
Sample preparation: Extract 20 μL with three 20 μL portions of acetone:diethyl ether 50:50. Combine the organic layer s and add 5 μL ethyl acetate, dry over 4 Å molecular sieve, evaporate to about 5 μL (mostly ethyl acetate), add 15 μg dansyl chloride, add 2 mg potassium carbonate, reflux for 2 h, dilute to 100 μL, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 500 × 2.5 30 μm pellicular C18
Mobile phase: Gradient. MeOH:water 0:100 for 8 min, to 65:35 over 20 min.
Flow rate: 1
Injection volume: 5
Detector: F ex 360 em 520

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Extracted: barbital, heptabarbital

KEY WORDS

derivatization; whole blood

REFERENCE

Dünges,W.; Naundorf,G.; Seiler,N. High pressure liquid chromatographic analysis of barbiturates in the picomole range by fluorometry of their DANS-derivatives, *J.Chromatogr.Sci.*, **1974**, *12*, 655–657.

SAMPLE

Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, centrifuge at 10000 g, dilute supernatant with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 110 × 4.7 5 μm PartiSphere C18 (Whatman)
Mobile phase: MeCN:15 mM pH 7.0 phosphate buffer 30:70
Flow rate: 0.8
Injection volume: 50
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted coil of PTFE tubing irradiated by an 8 W low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 4.4

OTHER SUBSTANCES

Extracted: butethal, pentobarbital, secobarbital

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Wolf,C.; Schmid,R.W. Enhanced UV-detection of barbiturates in HPLC analysis by on-line photochemical reaction, *J.Liq.Chromatogr.*, **1990**, *13*, 2207–2216.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 14.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: doxapram

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70, adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 5.8
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, mephobarbital, pentobarbital, phenobarbital, secobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 0.398

OTHER SUBSTANCES
Simultaneous: pentobarbital, amobarbital, butabarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine,

eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Aprotinin

Molecular formula: $C_{284}H_{432}N_{84}O_{79}S_7$
Molecular weight: 6511.53
CAS Registry No.: 9087-70-1
Merck Index: 796

SAMPLE

Matrix: blood, urine
Sample preparation: Prepare an affinity column with porcine pancreatic kallikrein bound to cyanogen bromide-activated Sepharose 4B according to the method of the manufacturer (Pharmacia). Use prolonged washing cycles of high and low pH. Immediately acidify urine to pH 2.0. Adjust pH of plasma to 8.3 with 100 mM pH 8.3 Tris-HCl buffer containing 500 mM NaCl. Adjust pH of urine to 8.3 with 2 M NaOH. Centrifuge sample at 4390 g for 10 min, pump onto affinity column, pump eluate twice onto column. Wash column with ten bed volumes 100 mM pH 8.3 Tris-HCl buffer containing 500 mM NaCl. Elute with 800 μL 1 M phosphoric acid and 800 μL 10 mM pH 2.2 phosphoric acid in 200 mM sodium perchlorate, mix, inject a 1000 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm LiChrosorb RP-18
Mobile phase: MeCN:200 mM pH 2.2 sodium perchlorate containing 10 mM phosphoric acid 30:70
Column temperature: 25
Flow rate: 1
Injection volume: 1000
Detector: UV 200

CHROMATOGRAM

Retention time: 9.3
Limit of detection: 50 ng/mL

KEY WORDS

plasma; affinity chromatography

REFERENCE

Raspi,G.; Lo Moro,A.; Spinetti,M. High-performance liquid chromatographic method for the determination of aprotinin in body fluids, *J.Chromatogr.*, **1990**, *525*, 426–432.

SAMPLE

Matrix: formulations
Sample preparation: Dilute (if necessary) to a concentration of 500 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco LC-304 (pore size 30 nm)
Mobile phase: MeCN:31 mM sodium perchlorate 22:78
Flow rate: 0.5
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Retention time: 35
Limit of detection: 5000 ng/mL

REFERENCE

Dimov,N.; Simeonov,S. Purity evaluation of aprotinin by high performance liquid chromatography, *Biomed.Chromatogr.*, **1993**, *7*, 146–148.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 100-1000 μL aliquot of buffer solution containing aprotinin.

HPLC VARIABLES

Column: 250 × 4.6 7 μm LiChrosorb RP-18
Mobile phase: MeCN:10 mM phosphoric acid in 200 mM sodium perchlorate 30:70
Column temperature: 25
Flow rate: 1
Injection volume: 100-1000
Detector: UV 200

CHROMATOGRAM

Retention time: 9.3
Limit of detection: 600 ng/mL

REFERENCE

Raspi,G.; Lo Moro,A.; Spinetti,M.; Molinari,M. Determination of aprotinin by titration with bovine trypsin with end-point detection by high-performance liquid chromatography, *Analyst*, **1989**, *114*, 1017–1019.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Vydac C4 (code 214TP54)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid and 2% ammonium sulfate. B was 0.07% trifluoroacetic acid in MeCN. A:B from 95:5 to 75:25 over 20 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 15.8

OTHER SUBSTANCES

Simultaneous: degradation products

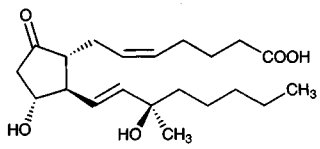
KEY WORDS

cow

REFERENCE

Vinther,A.; Bjorn,S.E.; Sorensen,H.H.; Soeberg,H. Identification of aprotinin degradation products by the use of high-performance capillary electrophoresis, high-pressure liquid chromatography and mass spectrometry, *J.Chromatogr.*, **1990**, *516*, 175–184.

Arbaprostil



Molecular formula: $C_{21}H_{34}O_5$
Molecular weight: 366.50
CAS Registry No.: 55028-70-1
Merck Index: 808
Lednicer No.: 3 8

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 200 mg and a 1 mL 100 mg Bond-Elut C18 SPE cartridge with 4 mL MeOH and 4 mL water. 3 mL Plasma + 12 μL 32.5 ng/mL IS in MeCN + 300 μL 5% formic acid, vortex, centrifuge at 4° at 1500 g for 15 min, add the supernatant to the 3 mL SPE cartridge, wash with two 2 mL portions of water, wash with two 2 mL portions of MeOH:water 10:90, wash with 2 mL toluene, elute with 1 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 610 μL MeOH, vortex for 30 s, add 1.42 mL 0.01% formic acid, inject a 1.8 mL aliquot onto a 30 × 4.6 5 μm Brownlee RP-8 column and elute to waste with MeCN:water:formic acid 40:60:0.01 at 2 mL/min, after 2.5 min backflush the contents of this column onto a 250 × 4.6 5 μm Supelcosil LC-18 column eluted with MeCN:water:formic acid 40:60:0.01 at 2 mL/min, after about 6.5-7 min collect a fraction containing the prostaglandins. Dilute this fraction with an equal volume of water, add to the 1 mL SPE cartridge, wash with 1 mL hexane, elute with two 500 μL portions of ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL 100 μg/mL panacyl bromide in THF:MeCN 20:80, vortex for 30 s, add 3 μL N,N-diisopropylethylamine, heat at 40° for 1 h (Anal. Chem. 1984, 56, 1866). Evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 230 μL isooctane:ethylene dichloride:isopropanol 70:30:1, sonicate for 10 min, inject a 200 μL aliquot onto column A and elute to waste with mobile phase A, after 3 min divert the effluent from column A onto column B and elute both to waste, after 1.5 min remove column A from the circuit, continue to elute column B to waste with mobile phase A, after 7.5 min collect the effluent from column B in a 2.2 mL sample loop, after 2 min inject the contents of this sample loop onto column C with mobile phase B, elute column C with mobile phase B, monitor the effluent from column C. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°) (Anal. Biochem. 1987, 165, 220).)

HPLC VARIABLES

Column: A 10 × 4.6 Co:Pell PAC; B 150 × 4.6 6 μm Zorbax CN; C 240 × 4.6 6 μm Zorbax Sil

Mobile phase: A Hexane:dichloromethane:isopropanol 70:30:1; B Hexane:dichloromethane:THF:isopropanol 60:20:20:1
Flow rate: 1
Injection volume: 200
Detector: F ex 375 em 470

CHROMATOGRAM
Retention time: 36 (arbaprostil), 39.5 (15S epimer)
Internal standard: 5,6-trans-(15R)-15-methylprostaglandin E_2 (U-67205) (37.5)
Limit of quantitation: 10 pg/mL

KEY WORDS
derivatization; SPE; plasma; column-switching; normal phase

REFERENCE
Pullen,R.H.; Cox,J.W. Determination of (15*R*)- and (15*S*)-15-methylprostaglandin E2 in human plasma with picogram per milliliter sensitivity by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 271–283.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Formulation + IS + 500 μL 2% phosphoric acid, mix, add 10 mL ethyl ether:chloroform 80:20, extract, centrifuge. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL 2.5 mg/mL p-nitrophenacyl bromide in MeCN, add 500 μL 12.5 μL/mL N,N-diisopropylethylamine in MeCN, vortex briefly, heat at 40° for 30 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, vortex, inject a 5-25 μL aliquot.

HPLC VARIABLES
Column: μPorasil silica gel
Mobile phase: MeCN:dichloromethane:water 30:70:0.5
Flow rate: 1.5
Injection volume: 5-25
Detector: UV 254

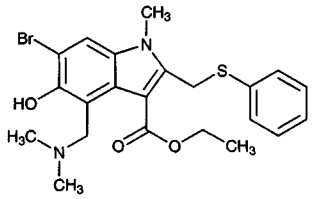
CHROMATOGRAM
Retention time: 6.8
Internal standard: 17β-hydroxy-17-methyl-4-androstene-3,11-dione (5.8)

OTHER SUBSTANCES
Simultaneous: s-epimer

KEY WORDS
derivatization; injections; normal phase; siliconise glassware with Surfasil (Pierce)

REFERENCE
Peng,G.W.; Sood,V.K. Liquid chromatographic assay of arbaprostil, *J.Liq.Chromatogr.*, **1983**, *6*, 1499–1511.

Arbidol



Molecular formula: $C_{22}H_{25}BrN_2O_3S$
Molecular weight: 477.42
CAS Registry No.: 131707-25-0, 131707-23-8 (HCl)

SAMPLE

Matrix: blood

Sample preparation: Vortex thawed plasma for 15 s, centrifuge at14 000 rpm for 1 min. Add 250 μL saturated sodium hydrogen carbonate to 1 mL plasma. Add 100 μL 5 μg/mL IS in MeCN and 5 mL MTBE. Shake at 50 rpm for 10 min and centrifuge at 4000 rpm for 10 min. Evaporate organic phase at 35° under a gentle flow of nitrogen. Reconstitute the residue in 200 μL mobile phase, centrifuge at 14000 rpm for 5 min. Add 200 μL hexane, mix for 15 sec, centrifuge at 14000 rpm for 3 min. Inject a 100 μL aliquot of the aqueous phase. (Protect sample from light!)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher RP 8
Column: 250 × 4.6 5 μm LiChrosorb RP 8
Mobile phase: MeOH:buffer 70:30 adjusted to pH 3.0 with orthophosphoric acid (Buffer was 5 mM heptanesulfonic acid containing 50 mM ammonium perchlorate and 1.32% (v/v) triethylamine.)
Column temperature: 35
Flow rate: 1.2
Injection volume: 100
Detector: UV 315

CHROMATOGRAM

Retention time: 5.3-5.4
Internal standard: SI 5 (G.D.Searle) (8.5-8.8)
Limit of quantitation: 5 ng/mL

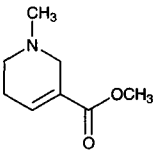
KEY WORDS

plasma

REFERENCE

Metz,R.; Muth,P.; Ferger,M.; Kukes,V.G.; Vergin,H. Sensitive high-performance liquid chromatographic determination of arbidol, a new antiviral compound in human plasma, *J.Chromatogr.A*, **1998**, *810*, 63–69.

Arecoline



Molecular formula: $C_8H_{13}NO_2$
Molecular weight: 155.20
CAS Registry No.: 63-75-2, 300-08-3 (HBr)
Merck Index: 815

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 3.1

KEY WORDS

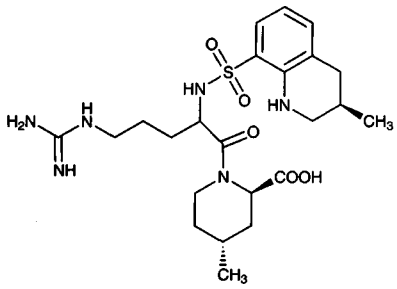
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Argatroban

Molecular formula: $C_{23}H_{36}N_6O_5S$
Molecular weight: 508.64
CAS Registry No.: 74863-84-6
Merck Index: 816



SAMPLE
Matrix: solutions

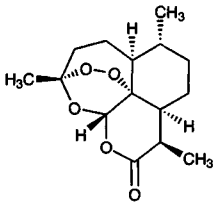
HPLC VARIABLES
Column: 250 × 4.6 Rainin 300Å C-18
Mobile phase: MeOH:water 33:67 containing 10 mM ammonium acetate
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 153 (R), 163 (S)

KEY WORDS
chiral

REFERENCE
Rawson,T.E.; VanGorp,K.A.; Yang,J.; Kogan,T.P. Separation of 21-(R)- and 21-(S)-argatroban: solubility and activity of the individual diastereoisomers [letter], *J.Pharm.Sci.*, **1993**, *82*, 672–673.

Artemisinin



Molecular formula: $C_{15}H_{22}O_5$
Molecular weight: 282.34
CAS Registry No.: 63968-64-9
Merck Index: 856

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 4 mL n-chlorobutane, vortex for 30 s, centrifuge at 5000 rpm for 15 min, freeze in acetone/dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH, add 400 μL 0.2% NaOH, mix, heat at 45° for 30 min, cool, add 50 μL 100 mM acetic acid in MeOH, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 100 mm long 5 μm LiChrosorb C18
Mobile phase: MeOH:10 mM pH 4.5 phosphate buffer 45:55
Flow rate: 0.8
Injection volume: 200
Detector: UV (wavelength not specified)

CHROMATOGRAM

Retention time: 10
Limit of detection: 2.5 ng/mL

KEY WORDS

serum; pharmacokinetics; derivatization

REFERENCE

Titulaer,H.A.C.; Zuidema,J.; Kager,P.A.; Wetsteyn,J.C.F.M.; Lugt,C.B.; Merkus,F.W.H.M. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers, *J.Pharm.Pharmacol.*, **1990**, *42*, 810–813.

SAMPLE

Matrix: blood
Sample preparation: Condition a 4 mm diameter Empore C18 SPE membrane with 0.5 mL MeOH and 0.5 mL water, do not allow to dry. Centrifuge 1 mL serum, add to SPE membrane, wash with 300 μL water, elute with 100 μL MeCN:water 65:35, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 2 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 50:50
Flow rate: 0.3
Injection volume: 50
Detector: Chemiluminescence in a fluorescence detector with no light source emission wavelength 425 nm. The effluent from the column mixed with reagent pumped at 0.5 mL/min and flowed through a convoluted mixing coil (1.1 mL dead volume) to the detector. (Reagent was 15 μg/mL luminol and 30 μg/mL hematin in 100 mM NaOH. Let stand for 30 min before use. Protect from light. Prepare daily.)

CHROMATOGRAM

Retention time: 7
Internal standard: dihydroartemisinin (5)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: arteether, artemether

KEY WORDS
serum; post-column reaction; SPE

REFERENCE
Green,M.D.; Mount,D.L.; Todd,G.D.; Capomacchia,A.C. Chemiluminescent detection of artemisinin. Novel endoperoxide analysis using luminol without hydrogen peroxide, *J.Chromatogr.A*, **1995**, *695*, 237–242.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL saturated NaCl solution, vortex for 5 s, add 5 mL isooctane:1-chlorobutane 45:55, vortex for 3 min, centrifuge at 1440 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL EtOH:water 50:50, let stand at 4° for 18 h, deoxygenate with a stream of nitrogen at 5 mL/min for 2 min (J. Chromatogr. 1983, 256, 323), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosphere 100 CN
Mobile phase: MeCN:50 mM acetic acid 15:85 adjusted to pH 5.0 with 1 M NaOH
Column temperature: 30
Flow rate: 1.5
Injection volume: 20
Detector: E, Bioanalytical Systems Model 200A, glassy carbon electrode, Ag/AgCl reference electrode, operated in reductive mode under a helium atmosphere

CHROMATOGRAM
Retention time: 16.1
Internal standard: artemisinin

OTHER SUBSTANCES
Extracted: artemether, dihydroartemisinin

KEY WORDS
plasma; treat glassware with 5% dichlorodimethylsilane; artemisinin is IS

REFERENCE
Navaratnam,V.; Mansor,S.M.; Chin,L.K.; Mordi,M.N.; Asokan,M.; Nair,N.K. Determination of artemether and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies, *J.Chromatogr.B*, **1995**, *669*, 289–294.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg Bond-Elut phenyl SPE cartridge with 1 mL MeOH and 1 mL 1 M acetic acid. Add 1 mL plasma to the SPE cartridge, wash with two 1 mL portions of 1 M acetic acid, wash with 1 mL MeOH:1 M acetic acid 20:80, elute with two 1 mL portions of ethyl acetate:butyl chloride 20:80. Remove any portion of aqueous phase from the eluate and evaporate the organic portion to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 50-75 μL aliquot.

HPLC VARIABLES
Guard column: Symmetry C8 (Waters)

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:buffer 50:50 (Buffer was 40 mL 1 M acetic acid and 60 mL 1 M sodium acetate per liter, pH 4.8.)
Flow rate: 0.7
Injection volume: 50-75
Detector: UV 290 following post-column reaction. The column effluent mixed with 1.2 M KOH MeOH:water 90:10 pumped at 0.3 mL/min and the mixture flowed through a 1 mL reaction coil (Waters) at 69° to the detector.

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: artesunic acid, α-dihydroartemisinin, β-dihydroartemisinin

KEY WORDS
artemisinin is IS; post-column reaction; plasma; SPE

REFERENCE
Batty,K.T.; Davis,T.M.; Thu,L.T.; Binh,T.Q.; Anh,T.K.; Ilett,K.F. Selective high-performance liquid chromatographic determination of artesunate and α- and β-dihydroartemisinin in patients with falciparum malaria, *J.Chromatogr.B*, **1996**, *677*, 345–350.

SAMPLE
Matrix: blood, saliva
Sample preparation: 1.5 mL Plasma or saliva + 500 μL 0.9% NaCl + 2.5 mL ethyl acetate, vortex, centrifuge at 2800 rpm for 3 min, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL EtOH, add 400 μL 0.2% NaOH, heat at 50° for 30 min, cool rapidly in water, wash twice with 500 μL aliquots of ethyl acetate, centrifuge, evaporate traces of ethyl acetate with a stream of air at room temperature, add 40 μL 2.5 M acetic acid in EtOH, make up to 500 μL with MeOH:water 20:80, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeOH:water 40:60 containing 10 mM Na_2HPO_4-NaH_2PO_4
Column temperature: 35 ± 1
Flow rate: 1.3
Injection volume: 200
Detector: UV 260

CHROMATOGRAM
Retention time: 12
Limit of detection: 2.5 ng/mL

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Zhao,S. High-performance liquid chromatographic determination of artemisinine (Qinghaosu) in human plasma and saliva, *Analyst*, **1987**, *112*, 661–664.

SAMPLE
Matrix: leaves
Sample preparation: Dry Artemisia leaves at 40° for 24 h, crush, reflux with 100 mL hexane for 15 min, filter, evaporate hexane to dryness under vacuum at 40°. Add 25 mL

MeCN to the residue, sonicate, filter (0.45 μm). Add 100 μL filtrate to 100 μL 1 mg/mL acetophenone in MeCN, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Brownlee C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 55:45 (Buffer was 8.3 g sodium acetate and 4 mL glacial acetic acid in 1 mL water, pH 5.1.)
Flow rate: 0.45
Injection volume: 50
Detector: UV 289 following post-column derivatization with 1 M KOH in MeOH:water 9:1 at 0.2 mL/min. The mixture flowed through a 4.4 × 0.5 mm (sic) knitted PTFE capillary held at 70°.

CHROMATOGRAM

Retention time: 16.5
Internal standard: acetophenone (11.5)
Limit of detection: 25 ng

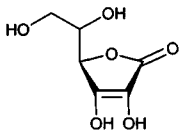
KEY WORDS

post-column reaction

REFERENCE

ElSohly,H.N.; Croom,E.M.; ElSohly,M.A. Analysis of the antimalarial sesquiterpene artemisinin in *Artemisia annua* by high-performance liquid chromatography (HPLC) with postcolumn derivatization and ultraviolet detection, *Pharm.Res.*, **1987**, *4*, 258–260.

Ascorbic acid



Molecular formula: $C_6H_8O_6$
Molecular weight: 176.13
CAS Registry No.: 50-81-7
Merck Index: 867

SAMPLE

Matrix: beverages, vegetables
Sample preparation: Blend 5 g food with 25 mL 300 mM trichloroacetic acid for 1 min, make up to 50 mL with 300 mM trichloroacetic acid. Dilute 5 g beverage to 50 mL with 300 mM trichloroacetic acid. Filter (paper) these solutions, dilute with 300 mM trichloroacetic acid to a ascorbic acidoncentration of 1-40 μg/mL. Remove a 3 mL aliquot ,and add it to 400 μL 4.5 M pH 6.2 sodium acetate buffer, add an ascorbate oxidase spatula (Boehringer Mannheim), heat at 37° for 2 min, mix, heat at 37° for 3 min, remove the spatula (?), add 500 μL 0.1% o-phenylenediamine (freshly prepared), mix, heat at 37° in the dark for 30 min, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long RP-18 (Bischoff)
Column: 125 × 4.6 3 μm ODS-Hypersil
Mobile phase: MeOH:80 mM KH_2PO_4 20:80, pH 7.8
Flow rate: 1
Injection volume: 30
Detector: F ex 365 (filter) em 418 (filter)

CHROMATOGRAM

Retention time: 8 (ascorbic acid), 10 (isoascorbic acid)
Limit of detection: 200 ng/g

KEY WORDS

derivatization; protect from light; avocado; brussels sprouts; cabbage; cauliflower; kale; lemon juice; lettuce; orange juice; paprika; parsley; peas

REFERENCE

Speek,A.J.; Schrijver,J.; Schreuers,W.H.P. Fluorometric determination of total vitamin C and total isovitamin C in foodstuffs and beverages by high-performance liquid chromatography with precolumn derivatization, *J.Agric.Food Chem.*, **1984**, *32*, 352–355.

SAMPLE

Matrix: beverages, vegetables
Sample preparation: 500 μL Juice or homogenized vegetables + 5 mg pyrogallol + 10 mL 100 mM citric acid, vortex under nitrogen for 1 min, add an equal volume of dichloromethane, vortex for 1 min, centrifuge at 4° at 1200 g for 10 min, repeat dichloromethane wash (if necessary to remove excess fat). Filter (0.45 μm) the aqueous layer, pass a 2 mL aliquot through a conditioned Sep-Pak C18 (?) SPE cartridge, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 5 DA-X8-11 anion-exchange resin (Dionex)
Mobile phase: 100 mM pH 3.8 Citrate buffer containing 10 mM NaCl and 5 mM EDTA
Flow rate: 0.5
Injection volume: 100
Detector: F ex Corning 7-60 filter em Wratten 2-E following post-column reaction. The column effluent mixed with the oxidizer pumped at 0.5 mL/min and this mixture flowed

through a 32 cm × 0.25 mm ID stainless-steel coil. The effluent from this coil mixed with the reagent pumped at 0.5 mL/min and this mixture flowed through a 45.7 m × 0.25 mm ID stainless-steel coil at 70° then a 1.5 m × 0.25 mm ID stainless-steel coil at 20° to the detector. (Oxidizer was 2.5 mM mercuric chloride or copper sulfate in mobile phase. Reagent was 3.1 mM o-phenylenediamine in mobile phase.)

CHROMATOGRAM
Retention time: 23
Limit of detection: 20 ng

OTHER SUBSTANCES
Extracted: dehydroascorbic acid

KEY WORDS
post-column reaction; bean sprouts; beets; broccoli; grape juice; orange juice; potatoes; tomatoes; SPE

REFERENCE
Vanderslice,J.T.; Higgs,D.J. HPLC analysis with fluorometric detection of vitamin C in food samples, *J.Chromatogr.Sci.*, **1984**, *22*, 485–489.

SAMPLE
Matrix: blood
Sample preparation: Dilute an aliquot of plasma or serum with an equal volume of 10% metaphosphoric acid, add IS to a final concentration of 4.5 μg/mL, centrifuge at 3300 g for 10 min, filter the supernatant (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS C18
Mobile phase: 50 mM pH 4.5 KH_2PO_4 containing 5 mM cetyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7
Internal standard: 4-hydroxyacetanilide (13.9)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: dehydroascorbic acid

KEY WORDS
plasma; serum

REFERENCE
Esteve,M.J.; Farré,R.; Frigola,A.; Garcia-Cantabella,J.M. Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography, *J.Chromatogr.B*, **1997**, *688*, 345–349.

SAMPLE
Matrix: blood
Sample preparation: Collect 5 mL whole blood in a tube with 100 μL glutathione solution. Remove a 1 mL aliquot and add it to 4 mL 300 mM trichloroacetic acid, vortex thoroughly for 10 min, let stand in the dark at 4° for 10 min, mix, let stand in the dark at 4° for 10 min, centrifuge at 4° at 2000 g for 10 min. Remove a 1.5 mL aliquot of the supernatant and add it to 200 μL 4.5 M pH 6.2 sodium acetate buffer, add an ascorbate oxidase spatula (Boehringer Mannheim), heat at 37° for 2 min, mix, heat at 37° for 3 min, remove the

spatula, add 250 μL 0.1% o-phenylenediamine (freshly prepared), mix, heat at 37° in the dark for 30 min, inject a 20 μL aliquot. (Prepare glutathione solution by dissolving 1.5 g glutathione in 25 mL water, adjust pH to 6.5 with 2 M NaOH, add 2.25 g ethyleneglycolbis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), adjust pH to 6.5 with 10 M NaOH.)

HPLC VARIABLES
Column: 80 × 4.6 3 μm ODS-Hypersil
Mobile phase: MeOH:80 mM KH_2PO_4 20:80, pH 7.8
Flow rate: 1
Injection volume: 20
Detector: F ex 365 (filter) em 418 (filter)

CHROMATOGRAM
Retention time: 3
Limit of detection: 200 nM

KEY WORDS
derivatization; protect from light; whole blood

REFERENCE
Speek,A.J.; Schrijver,J.; Schreurs,W.H. Fluorometric determination of total vitamin C in whole blood by high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1984**, *305*, 53–60.

SAMPLE
Matrix: blood
Sample preparation: Dilute plasma with an equal volume of cold 10% metaphosphoric acid containing 0.54 mM disodium EDTA, centrifuge, mix the supernatant with an equal volume of cold 5% metaphosphoric acid containing 0.54 mM disodium EDTA and 10 μg/mL isoascorbic acid, dilute 25 fold with cold 1.04 mM cysteine containing 0.54 mM EDTA, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. (Maintain at 4° during sample preparation and in autosampler.)

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 7.5:92.5 adjusted to pH 4.75 with glacial acetic acid (Buffer was 40 mM sodium acetate containing 0.54 mM disodium EDTA and 1.5 mM dodecyltrimethylammonium phosphate.) (At the end of each week wash column with 50-100 mL water and 50-100 mL MeOH, store in MeOH.)
Flow rate: 0.8
Injection volume: 50
Detector: E, Bioanalytical systems LC4B, glassy carbon working electrode, stainless steel electrode top, Ag/AgCl reference electrode, +0.5 V

CHROMATOGRAM
Retention time: 14.8
Internal standard: isoascorbic acid (16.1)
Limit of detection: 0.02 ng
Limit of quantitation: 0.2 ng

KEY WORDS
plasma

REFERENCE
Kutnink,M.A.; Hawkes,W.C.; Schaus,E.E.; Omaye,S.T. An internal standard method for the unattended high-performance liquid chromatographic analysis of ascorbic acid in blood components, *Anal. Biochem.*, **1987**, *166*, 424–430.

SAMPLE
Matrix: blood
Sample preparation: 20 μL Plasma + 10 μL 125 μg/mL α-methyl-L-dopa in water, add to a PCPure hydroxyapatite SPE cartridge (Moritex or Koken), elute with buffer, collect 800 μL eluate, inject a 20 μL aliquot. (Buffer was freshly prepared 10 mM pH 6.8 sodium phosphate buffer containing 2.5 mg/mL L-cysteine.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: 100 mM KH_2PO_4 containing 1 mM disodium EDTA, adjusted to pH 3 with phosphoric acid
Flow rate: 0.6
Injection volume: 20
Detector: E, Irica Σ875, 300 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: α-methyl-L-dopa (15)
Limit of detection: 240 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Iwase,H.; Ono,I. Determination of ascorbic acid in human plasma by high-performance liquid chromatography with electrochemical detection using a hydroxyapatite cartridge for precolumn deproteinization, *J.Chromatogr.B*, **1994**, *655*, 195–200.

SAMPLE
Matrix: blood
Sample preparation: Add one volume of 10% metaphosphoric acid to 3 volumes of plasma, freeze the clear supernatant at -80°, thaw, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 4 μm triacontyl Daltosil 100 C30 (Serva)
Mobile phase: 13.61 g/L KH_2PO_4 adjusted to pH 2.34 with concentrated orthophosphoric acid
Flow rate: 0.5 for 10 min then 1 for 5 min
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 9.9
Limit of detection: 2 ng

KEY WORDS
plasma

REFERENCE
Manoharan,M.; Schwille,P.O. Measurement of ascorbic acid in human plasma and urine by high-performance liquid chromatography. Results in healthy subjects and patients with idiopathic calcium urolithiasis, *J.Chromatogr.B*, **1994**, *654*, 134–139.

SAMPLE
Matrix: blood
Sample preparation: 600 μL Plasma + 600 μL 10% metaphosphoric acid, freeze, store at -80°, allow to thaw at 4° for 1 h, centrifuge at 4° at 1000 g for 10 min, inject a 20 μL aliquot of the supernatant

HPLC VARIABLES
Guard column: 20 × 4 30-40 μm Perisorb pellicular C18 (Anachem)
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:buffer 7.5:92.5, pH 5.5 (Buffer was 25 mM myristyltrimethylammonium bromide containing 50 mM NaOH, 60 mM acetic acid, 100 μg/mL homocysteine, and 200 μg/mL EDTA.)
Flow rate: 0.55
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 5.32

OTHER SUBSTANCES
Extracted: uric acid

KEY WORDS
plasma

REFERENCE
Ross,M.A. Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *657*, 197–200.

SAMPLE
Matrix: blood
Sample preparation: 30 μL Plasma + 60 μL stabilizing solution, mix, let stand on ice for 10-15 min, centrifuge at 4° at 12000 g for 5 min, inject a 1-5 μL aliquot of the supernatant. (Stabilizing solution was MeOH:water 90:10 saturated with EDTA.)

HPLC VARIABLES
Column: 250 × 4.5 QC Pack C18 (IRICA) or 250 × 4.5 TSK gel ODS 120A (Toyo Soda)
Mobile phase: MeOH:water 20:80 containing 50 mM sodium phosphate, 50 mM sodium acetate, 189 μm dodecyltrimethylammonium chloride, 36.6 μm tetraoctylammonium bromide, 0.2 mM EDTA, pH adjusted to 4.8 with phosphoric acid (Dissolve dodecyltrimethylammonium chloride in MeOH first.)
Flow rate: 1
Injection volume: 1-5
Detector: E, IRICA Sigma 875, +350 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3
Limit of detection: 0.1 ng

KEY WORDS
plasma

REFERENCE
Umegaki,K.; Inoue,K.; Takeuchi,N.; Higuchi,M. Improved method for the analysis of ascorbic acid in plasma by high-performance liquid chromatography with electrochemical detection, *J.Nutr. Sci.Vitaminol.(Tokyo)*, **1994**, *40*, 73–79.

SAMPLE

Matrix: blood, food, formula, perfusate

Sample preparation: Serum, perfusate. Vortex 1.5 mL plasma or perfusate with 160 μL 400 mg/mL metaphosphoric acid in water for 15 s, add 400 μL MeCN vortex for 15 s, centrifuge at 4° at 1000 g for 30 min, inject an aliquot (Clin.Chem. 1988, 34, 2217). Infant formula. Mix 5 g formula with 40 mL 50 mM monobasic potassium phosphate in water containing 1 g/L dithiothreitol, allow to dissolve. Add 10 mL 500 g/L metaphosphoric acid in water and 20 mL MeCN, mix, centrifuge at 1000 g for 15 min at 5°. Remove a 4 mL aliquot of the clear lower layer, dilute with 50 mM monobasic potassium phosphate in water containing 1 g/L dithiothreitol, inject an aliquot. Food (for total ascorbic acid). Suspend 1 g pureed food in 5 mL water, vortex for 15 s, add 1 mL 500 mM dibasic potassium phosphate in water containing 100 g/L dithiothreitol, vortex for 15 s, let stand at room temperature for 30 min, add 1 mL 400 g/L metaphosphoric acid in water, add 2 mL MeCN, vortex for 15 s, centrifuge at 1000 g for 30 min at 5°, inject an aliquot of the clear lower layer.

HPLC VARIABLES

Column: 250 × 4.6 Capcell Pak NH2 (Shiseido, Japan)

Mobile phase: MeCN:buffer 80:20 (Prepare mobile phase as follows. Dissolve 680 mg monobasic potassium phosphate in 200 mL water, add 800 mL MeCN and 7.5 mL concentrated phosphoric acid.)

Column temperature: 40

Flow rate: 1

Detector: E, Model 400 (EG & G, Princeton Applied Research, USA), 700 mV

CHROMATOGRAM

Retention time: 4.2

OTHER SUBSTANCES

Extracted: dithiothreitol, uric acid

KEY WORDS

human; serum; rat

REFERENCE

Margolis,S.A.; Schapira,R.M. Liquid chromatographic measurement of L-ascorbic acid and D-ascorbic acid in biological samples, *J.Chromatogr.B*, **1997**, *690*, 25–33.

SAMPLE

Matrix: blood, formulations

Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. 40 μL Plasma + 80 μL MeCN, mix for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrosorb RP-18

Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min

Flow rate: 1

Injection volume: 10

Detector: UV 270

CHROMATOGRAM
Retention time: 1.89
Internal standard: xanthine (4.56)
Limit of detection: 2.5 ng

OTHER SUBSTANCES
Extracted: folic acid, niacin, niacinamide, riboflavin, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 200 μL 10% metaphosphoric acid, mix, centrifuge at 0° at 6000 g for 10 min, filter (Millipore Molcut II), inject a 10 μL aliquot of the filtrate. Liver. Homogenize 500 mg liver with 10 mL 5% metaphosphoric acid, mix, centrifuge at 0° at 6000 g for 10 min, filter (Millipore Molcut II), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 7.9 10 μm Shimpack SCR-101H (Shimadzu)
Mobile phase: 5 mM oxalic acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 300 following post-column reaction. The column effluent mixed with 100 mM sodium borohydride in 100 mM NaOH pumped at 0.6 mL/min, the mixture flowed through a "j" type reaction coil (Shimadzu) maintained at 30° to the detector.

CHROMATOGRAM
Retention time: 16.8
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: dehydroascorbic acid

KEY WORDS
fish; carp; yellowtail; liver; plasma; post-column reaction

REFERENCE
Ito,T.; Murata,H.; Yasui,Y.; Matsui,M.; Sakai,T.; Yamauchi,K. Simultaneous determination of ascorbic acid and dehydroascorbic acid in fish tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *667*, 355–357.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 3 mL 10 g/L metaphosphoric acid, mix, centrifuge at 1500 g. Mix a 500 μL aliquot of the supernatant with 50 μL IS solution, inject a 20 μL aliquot. Urine. Dilute 1 mL urine with 3 mL 10 g/L metaphosphoric acid. Mix a 500 μL aliquot with 50 μL IS solution, inject a 20 μL aliquot. (IS solution was 100 mg/L D-isoascorbic acid in 10 g/L metaphosphoric acid containing 2 mM EDTA (nitrogen-saturated).)

HPLC VARIABLES

Guard column: 70 × 2 10 μm PRP-1 (Hamilton)
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: 0.15 mM Hexadecyltrimethylammonium bromide containing 0.5 mM sodium acetate and 0.15 mM disodium EDTA, adjusted to pH 4.0 with acetic acid. (Condition system with twenty 20 μL aliquots of 10 g/L metaphosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: F ex 365 em 440 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min and flowed through a 20 m × 0.55 mm ID PTFE coil at 65° then a 1.5 m × 0.55 mm ID PTFE coil at 20° to the detector. (Reagent was 2.5 mM cupric sulfate containing 52 mM citric acid and 0.5 mM 4,5-dimethyl-o-phenylenediamine dihydrochloride, adjusted to pH 4.1 with saturated NaOH. Prepare fresh each day. Prepare 4,5-dimethyl-o-phenylenediamine dihydrochloride by dissolving 4,5-dimethyl-o-phenylenediamine in a minimum volume of diethyl ether, pass anhydrous hydrogen chloride through the solution for 20 min, precipitate the salt with diethyl ether. Wash the salt three times with diethyl ether.)

CHROMATOGRAM

Retention time: 15.5
Internal standard: isoascorbic acid (19.9)
Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: dehydroascorbic acid, dehydroisoascorbic acid

KEY WORDS

post-column reaction; plasma

REFERENCE

Lopez-Anaya,A.; Mayersohn,M. Ascorbic and dehydroascorbic acids simultaneously quantified in biological fluids by liquid chromatography with fluorescence detection, and comparison with a colorimetric assay, *Clin.Chem.*, **1987**, *33*, 1874–1878.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 249.9

CHROMATOGRAM
Retention time: 2.928

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: food
Sample preparation: Blend food with 5% metaphosphoric acid containing L-cysteine, centrifuge at 4° at 57600 g for 10 min, filter (0.45 μm), immediately inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 C18 (Beckman)
Mobile phase: MeOH:water 75:25
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Limit of detection: 1.2 μg/mL

KEY WORDS
comparison with capillary electrophoresis; fruit; vegetables

REFERENCE
Choi,O.-K.; Jo,J.-S. Determination of L-ascorbic acid in foods by capillary zone electrophoresis, *J.Chromatogr.A*, **1997**, *781*, 435–443.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2

Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.3

OTHER SUBSTANCES
Simultaneous: niacinamide (UV 280), thiamine (UV 280), riboflavin (UV 280), pyridoxine (UV 280)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets and weigh out 1 g, add 1 mL formic acid, add 25 mL MeOH, shake mechanically for 10 min, make up to 50 mL with methanol. Remove 10 mL and centrifuge. 5 mL Supernatant + 5 mL 0.0025% p-hydroxybenzoic acid in MeOH:water 20:80, make up to 25 mL with water, inject an aliquot. (Analyze within 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb RP8
Mobile phase: MeOH:200 mM pH 3.5 phosphate buffer:water 20:10:70
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES
Simultaneous: aspirin, p-aminophenol, 3-O-acetylascorbic acid, 2-O-acetylascorbic acid, saccharin, acetaminophen, O-acetyl-p-aminophenol, salicylic acid (UV 280), diacetyl-p-aminophenol (UV 280)

KEY WORDS
tablets

REFERENCE
Thomis,R.; Roets,E.; Hoogmartens,J. Analysis of tablets containing aspirin, acetaminophen, and ascorbic acid by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1830–1833.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), niacinamide (UV 254), pyridoxamine, thiamine (UV 254), riboflavin (UV 254), pyridoxine

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, riboflavin, thiamine
Interfering: pyridoxine

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, 1993, **1993**,

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Juices. Dilute 2 mL fruit juice to 50 mL with 0.05% disodium EDTA in 100 mM sulfuric acid, filter (Whatman No. 1 paper), inject a 10 μL aliquot of the filtrate. Fruit, vegetables. Blend (Waring) fruit or vegetable with 0.05% disodium EDTA in 100 mM sulfuric acid for 3 min, centrifuge at 15000 rpm for 10 min. Remove the supernatant and make it up to 10 mL with 0.05% disodium EDTA in 100 mM sulfuric acid, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: MicroGuard ion exclusion cartridge (Bio-Rad)
Column: 300 × 7.8 Aminex HPX-87 (Bio-Rad)
Mobile phase: 4.5 mM Sulfuric acid
Flow rate: 0.5
Injection volume: 10
Detector: UV 245

CHROMATOGRAM
Retention time: 12.9
Limit of quantitation: 50 ng

KEY WORDS
juice; orange; lemon; grapefruit; pineapple; tomatoes; peas; potatoes; strawberries; green peppers

REFERENCE
Ashoor,S.H.; Monte,W.C.; Welty,J. Liquid chromatographic determination of ascorbic acid in foods, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 78–80.

SAMPLE
Matrix: juice
Sample preparation: 15 g Orange juice + 5 mL 12.5% trichloroacetic acid, centrifuge, filter, inject a 10 μL aliquot of the filtrate. (To determine ascorbic acid and dehydroascorbic acid (by reducing dehydroascorbic acid to ascorbic acid) dilute filtrate with water to an ascorbic acid concentration of 10-100 μg/mL adjust pH to 7.0. Remove a 500 μL aliquot and add it to 2 mL 0.8% DL-homocysteine, let stand for 15 min, filter, inject a 10 μL aliquot.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Erbasil NH2
Mobile phase: MeOH:0.25% pH 3.5 KH_2PO_4 50:50
Flow rate: 0.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7

KEY WORDS
orange juice; comparison with capillary electrophoresis

REFERENCE
Chiari,M.; Nesi,M.; Carrea,G.; Righetti,P.G. Determination of total vitamin C in fruits by capillary zone electrophoresis, *J.Chromatogr.*, **1993**, *645*, 197–200.

SAMPLE
Matrix: juice
Sample preparation: Dilute with water to a ascorbic acid concentration of 10 μg/mL, filter (0.45 μm), remove a 20 μL aliquot of the filtrate and add it to 10 μL 125 μg/mL α-methyl-L-dopa in water and 800 μL 2% metaphosphoric acid, inject a 20 μL aliquot. (To measure total ascorbic acid content after reduction of dehydroascorbic acid dilute juice with water to a ascorbic acid concentration of 10 μg/mL, filter (0.45 μm), remove a 20 μL aliquot of the filtrate and add it to 10 μL 125 μg/mL α-methyl-L-dopa in water and 800 μL buffer, let stand for 15 min, inject a 20 μL aliquot. (Buffer was freshly prepared 10 mM pH 6.8 sodium phosphate buffer containing 2.5 mg/mL L-cysteine.))

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: 100 mM KH_2PO_4 containing 1 mM disodium EDTA, adjusted to pH 3 with phosphoric acid
Flow rate: 0.6
Injection volume: 20
Detector: E, Irica Σ875, 300 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: α-methyl-L-dopa (15)
Limit of detection: 0.15 ng

KEY WORDS
lemon; grape; orange; apple; tea

REFERENCE
Iwase,H.; Ono,I. Determination of ascorbic acid and dehydroascorbic acid in juices by high-performance liquid chromatography with electrochemical detection using L-cysteine as precolumn reductant, *J.Chromatogr.A*, **1993**, *654*, 215–220.

SAMPLE
Matrix: plants
Sample preparation: Pulverize 200 mg plant tissue in liquid nitrogen, extract twice with a total volume of 3% metaphosphoric acid containing 1 mM EDTA, centrifuge, pass through a conditioned C18 SPE cartridge (Millipore), inject an aliquot of the last 500 μL of the eluate.; SPE

HPLC VARIABLES
Column: 250 × 4.6 3 μm C18 (Bio-Rad)

Mobile phase: pH 2.5 Phosphoric acid containing 0.1 mM EDTA
Flow rate: 0.8
Injection volume: 20
Detector: UV 248

CHROMATOGRAM
Retention time: 3.2

KEY WORDS
comparison with capillary electrophoresis; SPE

REFERENCE
Davey,M.W.; Bauw,G.; Montagu,M.V. Analysis of ascorbate in plant tissues by high-performance capillary zone electrophoresis, *Anal.Biochem.*, **1996**, *239*, 8–19.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Capcell Pak NH_2 (Shiseido, Japan)
Mobile phase: MeCN:buffer 80:20 (Prepare mobile phase as follows. Mix 680 mg monobasic potassium phosphate, 7.5 mL concentrated phosphoric acid with 200 mL water, add 800 mL MeCN.)
Column temperature: 40
Flow rate: 1.0
Detector: E, Model 400 (EG & G, Princeton Applied Research, Princeton, NJ), 700 mV

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: dithiothreitol, isoascorbic acid, metaphosphoric acid, uric acid,

REFERENCE
Margolis,S.A.; Duewer,D.L. Measurement of ascorbic acid in human plasma and serum: stability, intralaboratory repeatability, and interlaboratory reproducibility, *Clin.Chem.*, **1996**, *42*, 1257–1262.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: salicylic acid, benzoic acid, quinine, dihydroquinine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM

Retention time: 0.5

OTHER SUBSTANCES

Simultaneous: niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, niacinamide

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM

Retention time: 0.6

OTHER SUBSTANCES

Simultaneous: niacin, niacinamide, pyridoxine, riboflavin, thiamine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.21.

SAMPLE

Matrix: tissue
Sample preparation: Deproteinize heart tissue with ice-cold 600 mM perchloric acid, neutralize, centrifuge at 4° at 26890 g for 15 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 3 μm LC-18-T (Supelco)
Column: 150 × 4.6 3 μm LC-18-T (Supelco)
Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 1:99, pH 7.0. B was MeOH:100 mM KH_2PO_4 containing 2.8 mM tetrabutylammonium hydroxide 30:70, pH 5.5. A:B 100:0 for

12 min, to 60:40 over 2 min, to 56:44 over 11 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.2
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: adenosine, adenosine triphosphate

KEY WORDS
rat; heart

REFERENCE
Lazzarino,G.; Di Pierro,D.; Tavazzi,B.; Cerroni,L.; Giardina,B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography, *Anal.Biochem.*, **1991**, *197*, 191–196.

SAMPLE
Matrix: urine
Sample preparation: Prepare a column by suspending 200-400 mesh Dowex 50W-X8 resin in water and pouring it into a 100 × 7 column, allow to settle, wash with 10 mL 2 M HCl, wash with water until the washings are neutral. Mix urine with an equal volume of 5% metaphosphoric acid containing 0.5% β-thiodiglycol. Add a 1 mL aliquot to the column, wash with 3.95 mL 2 mM tartaric acid containing 0.05% β-thiodiglycol, collect all the effluent from the column in a tube containing 50 μL 5% disodium EDTA cooled in ice, filter (0.45 μm) the eluate, inject a 50-250 μL aliquot.

HPLC VARIABLES
Column: two 50 × 7.6 Asahipak GS-320 hydrophilic gel columns in series
Mobile phase: 2.25 g/L Tartaric acid containing 0.75 g/L disodium EDTA and 0.5 g/L β-thiodiglycol, adjusted to pH 3.00-3.03 with 4 M NaOH
Column temperature: 30
Flow rate: 1
Injection volume: 50-250
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with 20 mM benzamidine hydrochloride pumped at 0.36 mL/min and with 750 mM pH 10 potassium borate buffer containing 200 mM potassium sulfite pumped at 0.36 mL/min and the mixture flowed through a 50 m × 0.5 mm ID PTFE tube at 90° to the detector.

CHROMATOGRAM
Retention time: 48

OTHER SUBSTANCES
Extracted: isoascorbic acid
Simultaneous: dehydroascorbic acid, diketogluconic acid, diketogulonic acid

KEY WORDS
post-column reaction; SPE

REFERENCE
Seki,T.; Yamaguchi,Y.; Noguchi,K.; Yanagihara,Y. Determination of ascorbic acid in human urine by high-performance liquid chromatography coupled with fluorimetry after post-column derivatization with benzamidine, *J.Chromatogr.*, **1987**, *385*, 287–291.

SAMPLE
Matrix: urine
Sample preparation: Filter (paper), dilute 6 times with 0.05% metaphosphoric acid, inject an aliquot. Alternatively, dilute with an equal volume of 200 mM dithiothreitol, let stand at room temperature for 30 min, dilute three fold with 0.05% metaphosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Ultrasphere
Mobile phase: 13.61 g/L KH_2PO_4 adjusted to pH 2.34 with concentrated orthophosphoric acid
Flow rate: 0.5 for 10 min then 1 for 5 min
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 8
Limit of detection: 1.5 ng

REFERENCE
Manoharan,M.; Schwille,P.O. Measurement of ascorbic acid in human plasma and urine by high-performance liquid chromatography. Results in healthy subjects and patients with idiopathic calcium urolithiasis, *J.Chromatogr.B*, **1994**, *654*, 134–139.

SAMPLE
Matrix: wine
Sample preparation: Adjust pH of wine to 7-8 with potassium bicarbonate. Remove a 1 mL aliquot and add it to 1 mL 170 mM phenacyl bromide in acetone, add 1 mL 17 mM 18-crown-6 in acetone, add 1 mL acetone, heat in a boiling water bath for 75 min, cool, inject a 10 μL aliquot. (Recrystallize phenacyl bromide from n-heptane.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 250 × 4 7 μm RP-18 (Merck)
Mobile phase: Gradient. MeOH:water from 35:65 to 85:15 over 20 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

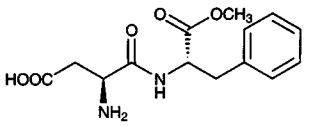
CHROMATOGRAM
Retention time: 11.1

OTHER SUBSTANCES
Extracted: acetic acid, anisic acid, benzilic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, salicylic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid

KEY WORDS
derivatization

REFERENCE
Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

Aspartame



Molecular formula: $C_{14}H_{18}N_2O_5$
Molecular weight: 294.31
CAS Registry No.: 22839-47-0
Merck Index: 874

SAMPLE
Matrix: beverages
Sample preparation: Filter sample.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Hiasil C18 (Higgins)
Mobile phase: MeOH:25 mM phosphate buffer 45:55, pH 3.0
Flow rate: 1.0
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 1.4 mg/mL

OTHER SUBSTANCES
Extracted: benzoic acid, caffeine

KEY WORDS
comparison with UV spectrophotometry and capillary electrophoresis; soft drinks

REFERENCE
McDevitt,V.L.; Rodriguez,A.; Williams,K.R. Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, *J.Chem.Educ.*, **1998**, *75*, 625–629.

SAMPLE
Matrix: beverages, formulations
Sample preparation: Dilute beverages and formulations with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Hexyl
Mobile phase: MeOH:0.1% perchloric acid 15:85, pH 2.8
Flow rate: 1
Detector: E, ESA Coulochem Model 5100A, first electrode (screen mode) +0.10 V, second electrode (measuring electrode) +0.80 V, following post-column reaction. The column effluent flowed through a 20 m × 0.3 mm ID PTFE coil irradiated with a UV 254 lamp to the detector.

CHROMATOGRAM
Retention time: 13
Limit of detection: 500 ng/mL
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: caffeine

KEY WORDS
post-column reaction; soft drinks; post-column photochemical derivatization

REFERENCE
Galletti,G.C.; Bocchini,P. High-performance liquid chromatography with electrochemical detection of aspartame with a post-column photochemical reactor, *J.Chromatogr.A*, **1996**, *729*, 393–398.

SAMPLE
Matrix: beverages, sweetener
Sample preparation: Sweetener. Dissolve 30 mg powdered tabletop sweetener in water and dilute to 25 mL, filter (0.2 μm PTFE). Beverages. Dilute fruit juice 1:10 with water. Degas carbonated beverages in a ultrasonic bath for 5 min, dilute 1:10 with water, filter. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 Dionex IonPak AG4A-SC
Column: 250 × 4 Dionex IonPak AS4A-SC
Mobile phase: Gradient. A was 1 mM sodium carbonate. B was 12.5 mM sodium carbonate. A:B 100:0 for 4.5 min, from 100:0 to 0:100 over 1 min, maintain at 0:100 for 22.5 min, from 0:100 to 100:0 over 0.1 min
Flow rate: 1
Injection volume: 50
Detector: UV 190 for 6 min, UV 206 22 min, then UV 190; Conductivity, Dionex ED40 in conductivity mode preceded by a Dionex ASRS-I suppressor (external water mode, 300 mA)

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 35 ng/mL (UV)

OTHER SUBSTANCES
Simultaneous: acesulfame, saccharin

REFERENCE
Chen,Q.-C.; Mou,S.-F.; Liu,K.-R.; Yang,Z.-Y.; Ni,Z.-M. Separation and determination of four artificial sweeteners and citric acid by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *771*, 135–143.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 9.8

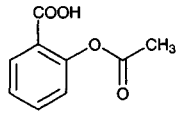
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Aspirin



Molecular formula: $C_9H_8O_4$
Molecular weight: 180.16
CAS Registry No.: 50-78-2
Merck Index: 886

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 200 mM orthophosphoric acid to 250 μL chilled plasma within 10 min of centrifuging (if fresh plasma) or within 10 min of thawing (if frozen plasma), vortex for 20 s, centrifuge at 5800 g for 3 min. Inject a 200 μL aliquot onto column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4.3 30 μm Hypersil C18 PEEK cartridge (Shandon, England); B 10 × 4 30 μm Hypersil C8 + 250 × 4.6 5 μm Nucleosil C8
Mobile phase: A Water:orthophosphoric acid 1000:1, pH 2.5; B MeCN:MeOH:water:orthophosphoric acid 150:200:650:1 (pH 2.6)
Flow rate: 1
Injection volume: 200
Detector: UV 225

CHROMATOGRAM

Retention time: 33
Limit of detection: 40 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: salicylic acid
Noninterfering: barbital, butobarbital, caffeine, 8-chlorotheophylline, clonazepam, cocaine, diazepam, flurazepam, furosemide, hydralazine, imipramine, nitrazepam, phenytoin, pindolol, propranolol, quinidine, theophylline
Interfering: xylazine, prazosin

KEY WORDS

column-switching; plasma

REFERENCE

McMahon,G.P.; Kelly,M.T. Determination of aspirin and salicylic acid in human plasma by column-switching liquid chromatography using on-line solid-phase extraction, *Anal.Chem.*, **1998**, *70*, 409–414.

SAMPLE

Matrix: formulation
Sample preparation: Weigh 500 mg homogenized analgesic powder, transfer to 100 mL volumetric flask, add ca. 50 mL mobile phase, swirl and dilute to volume with mobile phase. Dilute an aliquot of this solution 1:10 with mobile phase, filter (0.20 μm Cameo nylon filter, MSI, Westboro, MA) an aliquot, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 2.1 5 μm Hypersil ODS

Mobile phase: MeCN:triethylamine:acetic acid:water 5.5:0.2:0.2:94.1 (Prepare mobile phase as follows. Mix 110 mL MeCN, 4 mL triethylamine, 4 mL glacial acetic acid and make up to 2 L with water.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Extracted: acetaminophen, caffeine
Interfering: salicylic acid

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Add 50 mL of mobile phase to 0.5 g of sample and swirl to aid dissolution. Dilute to 100 mL with mobile phase. Dilute 1:10, filter (0.22 μm nylon). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:water:triethylamine:acetic acid 5.5:94.1:0.2:0.2
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Simultaneous: acetaminophen, caffeine

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 Scharlau Science, Spain
Mobile phase: MeOH:20 mM pH 4.KH_2PO_4 30:70 (pH adjusted with orthophosphoric acid)
Flow rate: 1.5
Injection volume: 135

Detector: UV 224

CHROMATOGRAM
Retention time: 12.8
Limit of quantitation: 3.6 μg/mL

OTHER SUBSTANCES
Simultaneous: caffeine, salicylic acid, thiamine

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out powdered sample containing 68 mg aspirin, add 80 mL MeOH, sonicate for 10 min, dilute to 100 mL with MeOH, centrifuge. Remove a 5 mL aliquot of the supernatant and add it to 1 mL 2 mg/mL resorcinol, add 2 mL MeOH, make up to 20 mL with 50 mM pH 3.0 triethylamine phosphate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: THF:50 mM pH 3.0 triethylamine phosphate 12:88
Flow rate: 0.6
Injection volume: 20
Detector: UV 275 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID crocheted PTFE coil irradiated with an 8 W low-pressure mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 15
Internal standard: resorcinol (9)

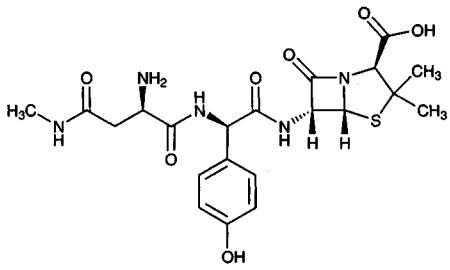
OTHER SUBSTANCES
Simultaneous: acetaminophen (post-column irradiation gives little increase in peak height), caffeine (post-column irradiation gives little increase in peak height), propyphenazone (post-column irradiation gives a decrease in peak height)

KEY WORDS
post-column reaction; post-column photochemical derivatization

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

Aspoxicillin

Molecular formula: $C_{21}H_{27}N_5O_7S$
Molecular weight: 493.54
CAS Registry No.: 63358-49-6
Merck Index: 887



SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4.)

HPLC VARIABLES

Column: 200 × 4 5 μm Nucleosil C18

Mobile phase: MeOH:buffer 8:92, adjusted to pH 5.8 with phosphoric acid (Buffer was 57.4 mM K_2HPO_4 adjusted to pH 5.8 with phosphoric acid.)

Flow rate: 1

Injection volume: 20-100

Detector: UV 220

CHROMATOGRAM

Retention time: 12

Limit of detection: 500 ng/mL

KEY WORDS

serum; plasma; lung; gut; pleural; chondral

REFERENCE

Knöller,J.; König,W.; Schönfeld,W.; Bremm,K.D.; Köller,M. Application of high-performance liquid chromatography of some antibiotics in clinical microbiology, *J.Chromatogr.*, **1988**, *427*, 257–267.

SAMPLE

Matrix: broncho-alveolar lavage fluid

Sample preparation: 1 mL Broncho-alveolar lavage fluid + 100 μL 5 μg/mL amoxicillin, vortex for 10 s, filter (Tosoh Ultracent-30 with a molecular mass cut-off at 30000) while centrifuging at 1500 g at 5° for 30 min, inject a 100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Shodex C18 5A (Showa Denko)

Mobile phase: MeCN:50 mM pH 3.0 potassium hydrogen phosphate containing 20 mM sodium 1-heptanesulfonate and 5 mg/L EDTA 10:100

Column temperature: 40

Flow rate: 1.2

Injection volume: 100

Detector: E, Irica Σ875, glassy carbon electrode 800 mV, Ag/AgCl reference electrode, following 10 m × 0.3 mm PTFE tubing irradiated by a GL-10 10 W mercury lamp

CHROMATOGRAM

Retention time: 24
Internal standard: amoxicillin (17)
Limit of detection: 1 ng/mL

KEY WORDS

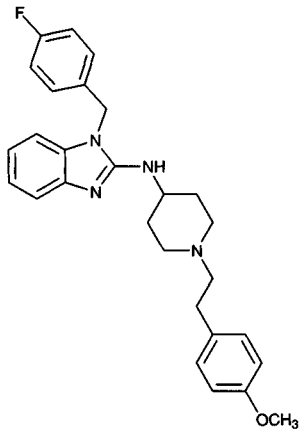
ultrafiltrate

REFERENCE

Yamazaki,T.; Ishikawa,T.; Nakai,H.; Miyai,M.; Tsubota,T.; Asano,K. Determination of aspoxicillin in broncho-alveolar lavage fluid by high-performance liquid chromatography with photolysis and electrochemical detection, *J.Chromatogr.*, **1993**, *615*, 180–185.

Astemizole

Molecular formula: $C_{28}H_{31}FN_4O$
Molecular weight: 458.58
CAS Registry No.: 68844-77-9
Merck Index: 891
Lednicer No.: 3 177



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.16

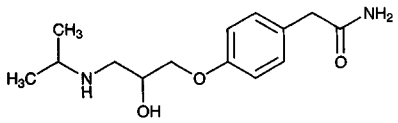
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Atenolol



Molecular formula: $C_{14}H_{22}N_2O_3$
Molecular weight: 266.34
CAS Registry No.: 29122-68-7
Merck Index: 892
Lednicer No.: 2 109

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 250 ng albuterol, mix for 10 s, add 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL mobile phase , mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: F ex 222 em 300

CHROMATOGRAM

Retention time: 15.7
Internal standard: albuterol (20.6)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: chlorthalidone, xipamide

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE

Matrix: blood
Sample preparation: Centrifuge plasma or serum at 11 300 g for 7 min, inject a 200 μL aliquot onto column A, elute to waste with mobile phase A, after 6 min backflush the contents of column A onto column B with mobile phase B, after 6 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Reequilibrate column A with mobile phase A for 4 min.)

HPLC VARIABLES

Column: A 20 × 4.0 BioTrap 500 C18 (ChromTech); B 12.5 × 4.6 5 μm Zorbax SB-CN + 150 × 4.6 5 μm Zorbax SB-CN
Mobile phase: A 2-Propanol:30 mM pH 7.0 sodium phosphate buffer containing 5 mM sodium octanesulfonic acid 2:98; B MeCN:30 mM pH 3.0 sodium phosphate buffer containing 2 mM sodium octanesulfonic acid 25:75

Flow rate: A 0.8; B 1
Injection volume: 200
Detector: F ex 230 em 300

CHROMATOGRAM
Retention time: 9.5

KEY WORDS
plasma; serum; column-switching

REFERENCE
Hermansson,J.; Grahn,A.; Hermansson,I. Direct injection of large volumes of plasma/serum on a new biocompatible extraction column for the determination of atenolol, propranolol and ibuprofen. Mechanisms for the improvement of chromatographic performance, *J.Chromatogr.A*, **1998**, *797*, 251–263.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma, whole blood. Condition a Bond-Elut C8 SPE cartridge with MeOH and water. Mix plasma or whole blood and 50 mM pH 9 borate buffer, add to the SPE cartridge. Wash with water and MeCN. Elute with MeOH. Evaporate the eluate to dryness and reconstitute in 400 μL mobile phase. Inject a 50μL aliquot. Tissue. Homogenize (Braun micro-dismembrator) 100 mg tissue with 400 μL water while frozen in liquid nitrogen, thaw, rinse twice with 250 μL 1 M pH 3 potassium phosphate buffer. Centrifuge at 2740 g at 20° for 20 min, separate supernatant (S1). Extract pellet with 1 mL MeOH for 15 min with sonication. Centrifuge at 20° for 10 min, evaporate the supernatant to dryness under a stream of nitrogen at 40°. Reconstitute residue in 500 μL 15 mM pH 3 potassium phosphate buffer add to S1, centrifuge. Suck sample slowly through a Bond-Elut C8 SPE cartridge. Wash twice with water, elute twice with 200 μL MeOH, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute in 500 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 17 × 4 5 μm Spherisorb C6
Column: 150 × 4.6 5 μm Spherisorb C6
Mobile phase: MeCN:15 mM pH 3 potassium phosphate buffer 17:83 (plasma) or 10:90 (tissue, blood)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 4.4 (plasma), 6.8 (whole blood, tissue)
Internal standard: atenolol

OTHER SUBSTANCES
Extracted: sotalol

KEY WORDS
whole blood; plasma; heart; SPE; atenolol is IS

REFERENCE
Laer,S.; Neumann,J.; Scholz,H.; Uebeler,P.; Zimmermann,N. Determination of sotalol in human cardiac tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 291–298.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.637

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1

Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.63, k' 2.24 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: perfusate
Sample preparation: Dilute perfusate 101 times with water and inject a 10 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water:acetic acid 19:80:1 containing 625 nM 1-heptane-sulfonic acid
Flow rate: 2
Injection volume: 10
Detector: F ex 225 em300

CHROMATOGRAM
Retention time: 2.1
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: metoprolol

KEY WORDS
rat

REFERENCE
Lindahl,A.; Krondahl,E.; Grudén,A.-C.; Ungell,A.-L.; Lennernäs,H. Is the jejunal permeability in rats age-dependent?, *Pharm.Res.*, **1997**, *14*, 1278–1281.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM pH 3.5 sodium phosphate buffer 15:85
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Dynamax C18 (Rainin)
Column: 250 × 4.6 5 μm Dynamax 300AC18 (Rainin)
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 10:90:0.1. B was MeCN:water:trifluoracetic acid 80:20:0.1. A:B from 100:0 to 70:30 over 12 min
Flow rate: 1
Detector: F ex 275 em 295

OTHER SUBSTANCES
Simultaneous: Tyr-containing peptides (F ex 278 em 305)

REFERENCE
Sorensen,M.; Steenberg,B.; Knipp,G.T.; Wang,W.; Steffansen,B.; Frokjaer,S.; Borchardt,R.T. The effect of β-turn structure on the permeation of peptides across monolayers of bovine brain microvessel endothelial cells, *Pharm.Res.*, **1997**, *14*, 1341–1348.

SAMPLE
Matrix: solutions
Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeCN:water 50:50
Flow rate: 1
Injection volume: 100
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM
Retention time: k' 5.2 (S-(-)), 6.1 (R-(+))

OTHER SUBSTANCES
Simultaneous: diacetolol

KEY WORDS
derivatization; chiral; F not much more sensitive than UV; $\alpha = 1.17$

REFERENCE
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: cimetidine, hydrochlorothiazide, ranitidine

KEY WORDS
tritium labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 × 3.2 5 μm Partisil ODS3
Column: 100 × 4.6 5 μm Partisil ODS3
Mobile phase: MeCN:buffer 10:90 (Buffer was 60 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.6-1
Injection volume: 10-100
Detector: UV 270

OTHER SUBSTANCES
Also analyzed: practolol

REFERENCE
Palm,K.; Luthman,K.; Ungell,A.-L.; Strandlund,G.; Artursson,P. Correlation of drug absorption with molecular surface properties, *J.Pharm.Sci.*, **1996**, *85*, 32–39.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Vydac C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 95:5 to 65:35 over 9 min.
Column temperature: 40

Flow rate: 1
Detector: UV (wavelength not given)

OTHER SUBSTANCES

Simultaneous: dexamethasone

REFERENCE

Rubas,W.; Cromwell,M.E.M.; Shahrokh,Z.; Villagran,J.; Nguyen,T.-N.; Wellton,M.; Nguyen,T.-H.; Mrsny,R.J. Flux measurements across Caco-2 monolayers may predict transport in human large intestinal tissue, *J.Pharm.Sci.*, **1996**, *85*, 165–169.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry

over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 35:65:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 26.7, 32.8 (enantiomers)
Limit of detection: 96-116 fmole

OTHER SUBSTANCES

Also analyzed: carteolol, timolol

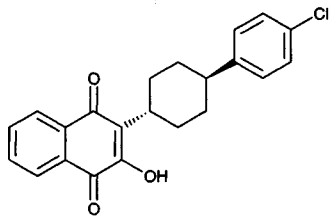
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Atovaquone



Molecular formula: $C_{22}H_{19}ClO_3$
Molecular weight: 366.84
CAS Registry No.: 95233-18-4
Merck Index: 898

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 1 mL phosphate buffer + 5 mL hexane:isoamyl alcohol 98:2, shake for 15 min, centrifuge at 1000 g for 5 min. Remove 2-4 mL organic layer and add it to 1 mL isopropanol, evaporate under a stream of nitrogen, dissolve in 200 μL MeOH:1% acetic acid 80:20, vortex for 10 s, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 Keystone C1 guard column
Column: 150 × 4.6 5 μm Supelcosil LC-1
Mobile phase: MeOH:1% acetic acid 70:30
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: pentamidine, zidovudine, trimethoprim, sulfamethoxazole

KEY WORDS

plasma

REFERENCE

DeAngelis,D.V.; Long,J.D.; Kanics,L.L.; Woolley,J.L. High-performance liquid chromatographic assay for the measurement of atovaquone in plasma, *J.Chromatogr.B*, **1994**, *652*, 211–219.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 1 mL 50 mM pH 7.0 KH_2PO_4/NaOH buffer + 5 mL hexane:3-methyl-1-butanol 98:2, tumble mix for 11 min, centrifuge at 1500 rpm for 11 min. Remove a 2 mL aliquot of the organic layer and add it to 800 μL isopropanol and 200 μL 100 ng/mL IS in isopropanol, evaporate under reduced pressure at 5 psi at 50° for 11 min, reconstitute with 500 μL hexane:3-methyl-1-butanol 98:2, evaporate for 11 min, reconstitute with 200 μL MeOH:1% pH 3.1 acetic acid 80:20, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 reverse phase stainless steel column (Chrompack)
Column: two 100 × 3 5 μm Chromspher C8 glass columns in series (Chrompack)
Mobile phase: MeCN:0.4% pH 2.0 trifluoroacetic acid 65:35
Flow rate: 0.6
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 9

Internal standard: trans-2-hydroxy-3-(4-phenylcyclohexyl)-1,4-naphthalenedione (59C80) (7)
Limit of quantitation: 250 ng/mL

KEY WORDS
plasma

REFERENCE
Studenberg,S.D.; Long,J.D.; Woolf,J.H.; Bruner,C.J.; Wilson,D.; Woolley,J.L. A robotics-based liquid chromatographic assay for the measurement of atovaquone in plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1383–1393.

SAMPLE
Matrix: blood
Sample preparation: Heat plasma at 56° for 1 h. 200 μL Plasma + 50 μL 100 μg/mL IS in MeOH:DMF 99:1, mix well, add 400 μL MeCN:1% aqueous acetic acid 85:15, mix, centrifuge at 14000 g for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C6
Mobile phase: MeOH:0.2% pH 2 aqueous trifluoroacetic acid containing 10 mM triethylamine 76:24
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.8
Internal standard: trans-2-hydroxy-3-(4-phenylcyclohexyl)-1,4-naphthalenedione (Burroughs-Wellcome) (7.4)
Limit of detection: 500 ng/mL

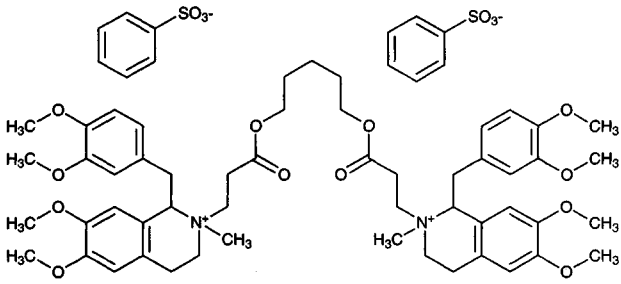
OTHER SUBSTANCES
Simultaneous: clindamycin, pentamidine, pyrimethamine, trimethoprim
Noninterfering: clarithromycin, dapsone, didanosine, fluconazole, folinic acid, foscarnet, gangiclovir, sulfadiazine, sulfamethoxazole, trimetrexate, zalcitabine, zidovudine

KEY WORDS
plasma

REFERENCE
Hansson,A.G.; Mitchell,S.; Jatlow,P.; Rainey,P.M. Rapid high-performance liquid chromatographic assay for atovaquone, *J.Chromatogr.B*, **1996**, *675*, 180–182.

Atracurium besylate

Molecular formula: $C_{65}H_{82}N_2O_{18}S_2$
Molecular weight: 1243.50
CAS Registry No.: 64228-81-5
Merck Index: 900



SAMPLE

Matrix: blood
Sample preparation: 250 µL Plasma + 250 µL picric acid (1:50 dilution of saturated picric acid solution) + 250 µL alcuronium solution + 250 µL water + 2.5 mL dichloromethane: isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 µL MeCN:water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 µPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: UV 210

CHROMATOGRAM

Retention time: 3.5
Internal standard: alcuronium (4.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Also analyzed: tubocurarine, metocurine

KEY WORDS

plasma

REFERENCE

Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE

Matrix: blood
Sample preparation: Adjust pH of plasma to 4 with 2 M sulfuric acid. 250 µL Acidified plasma + 10 µL 0.5 M sulfuric acid + 50 µL 5 µg/mL verapamil in water, mix, add 600 µL MeCN, vortex for 1 min, centrifuge at 2400 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.9 5 µm Spherisorb C8

Mobile phase: Gradient. A was MeCN:MeOH:30 mM K_2HPO_4 37.5:5:57.5, adjust final pH to 5. B was MeCN:MeOH:100 mM K_2HPO_4 37.5:15:47.5, adjust final pH to 5. A:B 0:100 to 100:0 over 8 min.
Flow rate: 1.7
Injection volume: 50
Detector: F ex 240 em 320

CHROMATOGRAM
Retention time: 6.2
Internal standard: verapamil (4)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: laudanosine

KEY WORDS
plasma

REFERENCE
Varin,F.; Ducharme,J.; Besner,J.G.; Théorêt,Y. Determination of atracurium and laudanosine in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 319–327.

SAMPLE
Matrix: blood
Sample preparation: Condition a Waters C18 SPE cartridge with 4 mL MeOH and 8 mL water. 500 µL Plasma + 2 mL 15 mM sulfuric acid, add a 500 µL aliquot to the SPE cartridge, wash with 2 mL 5 mM PIC B-8, elute with 1.5 mL MeOH containing 5 mM PIC B-8, inject an aliquot of the eluate.

HPLC VARIABLES
Guard column: C-1 (Keystone)
Column: 150 × 4.6 Spherisorb C-1
Mobile phase: Gradient. A was water containing 5 mM octanesulfonic acid (PIC B-8). B was MeCN:water containing 5 mM octanesulfonic acid (PIC B-8). A:B 52:48 for 8 min, then A:B 12:88 for 9.5 min (step gradient), re-equilibrate at initial conditions for 4.5 min (step gradient).
Flow rate: 1
Detector: F ex 202 em 320

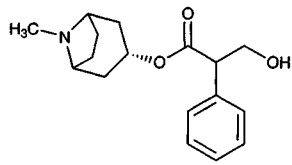
CHROMATOGRAM
Retention time: 15.5 (cis isomer)
Limit of detection: <40 nM

OTHER SUBSTANCES
Extracted: metabolites, laudanosine

KEY WORDS
human; rat; plasma; SPE

REFERENCE
Welch,R.M.; Brown,A.; Ravitch,J.; Dahl,R. The in vitro degradation of cisatracurium, the *R, cis-R'*-isomer of atracurium, in human and rat plasma, *Clin.Pharmacol.Ther.*, **1995**, *58*, 132–142.

Atropine



Molecular formula: $C_{17}H_{23}NO_3$
Molecular weight: 289.37
CAS Registry No.: 51-55-8, 52-88-0 (atropine methylnitrate)
Merck Index: 907
Lednicer No.: 1 35, 71, 93; 2 71

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 5 ng/mL scopolamine in MeOH, vortex briefly, add 50 μL 1 M ammonium hydroxide, mix, add 5 mL dichloromethane, shake horizontally for 5 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm BDS C18 (Keystone)
Column: 50 × 3 3 μm BDS C18 (Keystone)
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 62.5:37.5:15
Flow rate: 0.5
Injection volume: 20
Detector: MS, Perkin Elmer Sciex API III-Plus triple quadrupole, APCI, nebulizer 400° and 80 psi, auxiliary nitrogen 1.2 L/min, curtain gas 1.2 L/min, interface 55°, collision gas argon, electron multiplier 3000 V, declustering potential 35 V, collision energy 35 eV

CHROMATOGRAM

Retention time: 1.2
Internal standard: scopolamine (0.8)
Limit of quantitation: 20 pg/mL

KEY WORDS

plasma; protect from light

REFERENCE

Xu,A.; Havel,J.; Linderholm,K.; Hulse,J. Development and validation of an LC/MS/MS method for the determination of L-hyoscyamine in human plasma, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 33–42.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.388

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, plants
Sample preparation: Place 0.5 g powdered crude drug in 25 mL mobile phase, reflux 30 min, cool, centrifuge at 1600 g, decant wash residue twice with 10 mL portions of mobile phase, combine extracts and washings, make up to 50 mL with mobile phase, inject 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm TSK gel 120A ODS
Mobile phase: MeCN:67 mM pH 2.5 phosphate buffer 35:65 containing 17.5 mM sodium dodecylsulfate
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: scopolamine

REFERENCE
Oshima,T.; Sagara,K.; Tong,Y.Y.; Zhang,G.; Chen,Y.H. Application of ion-pair high performance liquid chromatography for analysis of hyoscyamine and scopolamine in solanaceous crude drugs, *Chem.Pharm.Bull.(Tokyo)*, **1989**, *37*, 2456–2458.

SAMPLE
Matrix: formulations
Sample preparation: Oral solutions. Dilute an amount oral solution equivalent to 25 μg atropine sulfate to 10 mL with EtOH. Tablets. Weight out finely powdered tablets equivalent to 25 μg atropine sulfate, add 10 mL MeCN:water 50:50, sonicate with frequent swirling for 15 min. Filter (0.45 μm membrane filter), discard the first portion. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: A:B 34:66 (Prepare A as follows. Dissolve 192 mg 1-pentanesulfonic acid sodium salt monohydrate in 200 mL water. Add 800 mL water and 1 mL orthophosphoric

acid. Prepare B as follows. Dissolve 192 mg 1-pentanesulfonic acid sodium salt monohydrate in 200 mL water. Add 800 mL MeCN and 1 mL orthophosphoric acid.)
Flow rate: 1.7
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: metabolites, diphenoxylate

KEY WORDS
oral solutions; tablets

REFERENCE
Lehr,G.J. Determination of diphenoxylate hydrochloride and atropine sulfate in combination drug formulations by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 1288–1293.

SAMPLE
Matrix: formulations
Sample preparation: Tablets, capsules. Powder tablets or remove contents of capsules, weigh out amount equivalent to about 600 μg hyoscyamine sulfate-atropine sulfate, add 25 mL 25 mM sulfuric acid, shake for 15 min, centrifuge at 3000 rpm for 5 min. Remove 5 mL of the supernatant and extract it twice with 30 mL portions of dichloromethane, discard the organic phase, add 2 mL buffer to the aqueous phase, extract with four 30 mL portions of dichloromethane, filter extracts through dichloromethane-rinsed glass wool, add 3 mL 2.25 μg/mL theophylline in dichloromethane, distil off the dichloromethane through a Snyder column by using a steam bath, when the volume reaches 10 mL rinse the column with 1-2 mL dichloromethane, continue distillation to 0.5-1 mL, remove the column and rinse the concentrator tube-column junction with 1 mL dichloromethane, evaporate to 1 mL with a stream of air at 40°, add 100 μL 1% concentrated HCl in MeOH, mix, evaporate to dryness with a stream of air at 40°, rinse the sides of the concentrator tube with 500 μL MeOH, evaporate to dryness with a stream of air at 40°, dissolve the residue in 300 μL water, inject a 20 μL aliquot. Elixirs. Add an amount equivalent to about 600 μg hyoscyamine sulfate-atropine sulfate to a 150 mL beaker, warm at 40° with a current of air for 30 min to remove alcohol, cool, make up to 25 mL with water, remove 5 mL of this solution, add 2 mL 100 mM sulfuric acid, extract twice with 30 mL portions of dichloromethane, discard the organic phase, add 2 mL buffer to the aqueous phase, extract with four 30 mL portions of dichloromethane, filter extracts through dichloromethane-rinsed glass wool, add 3 mL 2.25 μg/mL theophylline in dichloromethane, distil off the dichloromethane through a Snyder column by using a steam bath, when the volume reaches 10 mL rinse the column with 1-2 mL dichloromethane, continue distillation to 0.5-1 mL, remove the column and rinse the concentrator tube-column junction with 1 mL dichloromethane, evaporate to 1 mL with a stream of air at 40°, add 100 μL 1% concentrated HCl in MeOH, mix, evaporate to dryness with a stream of air at 40°, rinse the sides of the concentrator tube with 500 μL MeOH, evaporate to dryness with a stream of air at 40°, dissolve the residue in 300 μL water, inject a 20 μL aliquot. (Buffer was 5.3 g anhydrous sodium carbonate and 4.2 g sodium bicarbonate in 100 mL water, pH 9.4. Pass dichloromethane through 75 g basic aluminum oxide, Brockmann Activity Grade 1, store over 25 g alumina/4 L.)

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS
Mobile phase: MeOH:buffer 250:525 (The 50 mM tetramethylammonium phosphate buffer was prepared from 500 mL water + 23 mL 20% tetramethylammonium hydroxide in MeOH + 10 mL concentrated phosphoric acid, adjust to pH 2.0 with concentrated phosphoric acid, make up to 1 L with water.)

Flow rate: 0.8
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5
Internal standard: theophylline (6.5)

OTHER SUBSTANCES
Simultaneous: phenobarbital, scopolamine, atropine

KEY WORDS
tablets; capsules; elixirs

REFERENCE
Pennington,L.J.; Schmidt,W.F. Belladonna alkaloids and phenobarbital combination pharmaceuticals analysis I: High-performance liquid chromatographic determinations of hyoscyamine-atropine and scopolamine, *J.Pharm.Sci.*, **1982**, *71*, 951–953.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out amount containing 25 μg atropine, extract with 40 mL chloroform, filter, wash filter with chloroform, make up filtrate to 50 mL with chloroform, mix. Remove a 1 mL aliquot and evaporate it to dryness under reduced pressure at 40°, reconstitute with 500 μL 4 mg/mL quinuclidine in acetone, add 500 μL 2 mg/mL 1-anthroylnitrile in acetone, heat at 30° for 10 min, add 1 mL 2% phosphoric acid, cool to room temperature, make up to 10 mL with acetone, inject a 10 μL aliquot. (1-Anthroylnitrile is available from Wako Chemicals, Richmond VA. Synthesis is as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroylnitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmocil 5C-18 (Nacalai Tesque, Tokyo)
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM sodium dodecyl sulfate adjusted to pH 3.5 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 255 em 474

CHROMATOGRAM
Retention time: 12
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: albumin, amomum seed, caffeine, chlorpheniramine, cinnamon bark, cloves, fennel, geranium herb, glycyrrhiza, lysozyme, swertia herb, thiamine, riboflavin

KEY WORDS
derivatization; tablets

REFERENCE
Takahashi,M.; Nagashima,M.; Shigeoka,S.; Nishijima,M.; Kamata,K. Determination of atropine in pharmaceutical preparations by liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1997**, *775*, 137–141.

SAMPLE
Matrix: plants
Sample preparation: Dissolve alkaloids in 1 mL MeOH, add 40 ng homatropine, inject aliquot.

HPLC VARIABLES
Column: 150 × 4.1 5 μm Hamilton PRP-1
Mobile phase: MeCN:100 mM pH 10.4 ammonium acetate
Flow rate: 1
Injection volume: 20
Detector: MS thermospray, VG Trio-2, ion source 150°, vaporizer tip 170°, repeller electrode 150 V, m/z 290

CHROMATOGRAM
Internal standard: homatropine (m/z 276)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: scopolamine

KEY WORDS
total run time 6 min

REFERENCE
Auriola,S.; Martinsen,A.; Oksman-Caldentey,K.M.; Naaranlahti,T. Analysis of tropane alkaloids with thermospray high-performance liquid chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *562*, 737–744.

SAMPLE
Matrix: plants
Sample preparation: Extract 0.1 g dry plant material with 10 mL MeOH for 10 min under reflux, filter, inject aliquot.

HPLC VARIABLES
Guard column: 40 × 4 10 μm Hypersil ODS
Column: 250 × 4 10 μm Hypersil ODS
Mobile phase: MeOH:water 45:55 containing 0.1% phosphoric acid adjusted to pH 7 with triethylamine
Flow rate: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 20.7

OTHER SUBSTANCES
Simultaneous: scopolamine

REFERENCE
Hagemann,K.; Piek,K.; Stöckigt,J.; Weiler,E.W. Monoclonal antibody-based enzyme immunoassay for the quantitative determination of the tropane alkaloid, scopolamine, *Planta Med.*, **1992**, *58*, 68–72.

SAMPLE
Matrix: plants
Sample preparation: 100 mg Freeze-dried powdered plant leaves + 10 mL mobile phase, heat at 40° for 15 min, filter, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 4 μm Novapack C18
Mobile phase: MeCN:water 12.5:87.5 with 0.3% phosphoric acid adjusted to pH 2.2 with triethylamine
Flow rate: 0.8
Injection volume: 20
Detector: UV 204

CHROMATOGRAM
Retention time: 11.7
Limit of detection: 50000 ng/g

OTHER SUBSTANCES
Simultaneous: tropic acid, scopolamine

REFERENCE
Fliniaux,M.-A.; Manceau,F.; Jacquin-Dubreuil,A. Simultaneous analysis of l-hyoscyamine, l-scopolamine and dl-tropic acid in plant material by reversed phase high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *644*, 193–197.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

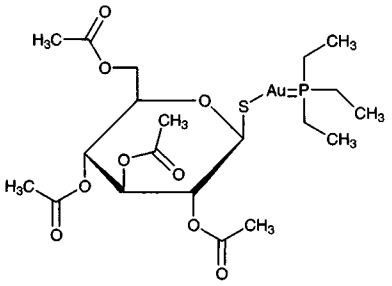
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 4.76

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Auranofin

Molecular formula: $C_{20}H_{34}AuO_9PS$
Molecular weight: 678.49
CAS Registry No.: 34031-32-8
Merck Index: 911



SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 or 0.22 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm octadecylsilane (Jones Chromatography)
Mobile phase: MeOH:0.25% ammonium dihydrogen phosphate 65:35
Flow rate: 0.8
Injection volume: 20-50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 0.2 ppm

KEY WORDS
Krebs-Ringer bicarbonate buffer

REFERENCE
Tepperman,K.; Finer,R.; Donovan,S.; Elder,R.C.; Doi,J.; Ratliff,D.; Ng,K. Intestinal uptake and metabolism of auranofin, a new oral gold-based antiarthritis drug, *Science*, **1984**, *225*, 430–432.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeOH:water 50:50 containing 10 mM tetrabutylammonium chloride and 25 mM ammonium formate, pH 6.3
Column temperature: 30
Flow rate: 1
Detector: UV 227 or MS, Sciex Elan 250 ICP-MS, monitor gold 197, RF power 1.4 kW, nebulizer Ar gas flow rate 1 L/min, nebulizer spray chamber 17

CHROMATOGRAM
Retention time: 18
Limit of detection: 0.3 ng

OTHER SUBSTANCES
Also analyzed: myochrysine

REFERENCE
Zhao,Z.; Jones,W.B.; Tepperman,K.; Dorsey,J.G.; Elder,R.C. Determination of gold-based antiarthritis drugs and their metabolites in urine by reversed-phase ion-pair chromatography with ICP-MS detection, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 279–287.

SAMPLE

Matrix: urine
Sample preparation: Filter (0.45 μm), inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 5 μm Spherisorb ODS2
Column: 150 × 4.6 5 μm B & J OD5 octadecyl
Mobile phase: MeOH:water 50:50 containing 10 mM tetrabutylammonium chloride and 25 mM ammonium formate, pH 6
Column temperature: 30
Flow rate: 1
Injection volume: 200
Detector: MS, Sciex Elan 250 ICP-MS, monitor gold 197, RF power 1.4 kW, nebulizer Ar gas flow rate 1 L/min, nebulizer spray chamber 17

CHROMATOGRAM

Retention time: 18
Limit of detection: 500 ng/mL

REFERENCE

Zhao,Z.; Jones,W.B.; Tepperman,K.; Dorsey,J.G.; Elder,R.C. Determination of gold-based antiarthritis drugs and their metabolites in urine by reversed-phase ion-pair chromatography with ICP-MS detection, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 279–287.

SAMPLE

Matrix: urine
Sample preparation: Rabbit urine. Centrifuge at 1000 g at 3° for 1 min, inject an aliquot. Human urine. Inject directly.

HPLC VARIABLES

Column: 150 × 4.6 YMC AM-302 octadecylsilyl (YMC)
Mobile phase: MeOH:water 65:35
Flow rate: 1
Injection volume: 100
Detector: UV 412 with post-column reaction detection. Reagent was 50 μM 5,5'-dithiobis(2-nitrobenzoic acid), 300 mM KI, and 50 mM pH 7.4 phosphate buffer delivered at 0.5 mL/min, mixed with column effluent, passed though a 0.5 mm × 5 m PTFE reaction coil at 60°.

CHROMATOGRAM

Retention time: 6

KEY WORDS

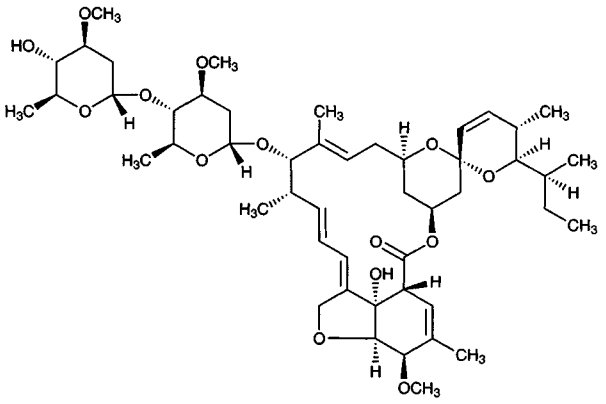
human; rabbit; post-column reaction

REFERENCE

Kizu,R.; Kaneda,M.; Yamauchi,Y.; Miyazaki,M. Determination of auranofin, a chrysotherapy agent, in urine by HPLC with a postcolumn reaction and visible detection, *Chem.Pharm.Bull.*, **1993**, *41*, 1261–1265.

Avermectin

Molecular formula: $C_{48}H_{74}O_{14}$
Molecular weight: 875.11
Merck Index: 919



SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

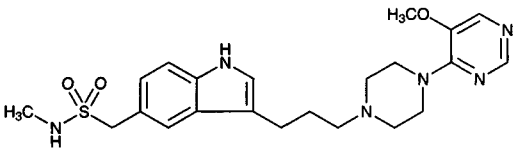
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, na-

lorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Avitriptan



Molecular formula: $C_{22}H_{30}N_6O_3S$
Molecular weight: 458.59
CAS Registry No.: 151140-96-4, 171171-42-9 (fumarate)

SAMPLE

Matrix: blood
Sample preparation: Add 50μL 1 M pH 5 ammonium acetate and IS to 500 μL plasma, mix. Add to a conditioned carboxylic acid BondElut SPE cartridge. Wash with pH 5.0 ammonium acetate and dichloromethane. Elute with 2 mL 1% triethylamine in MeOH. Evaporate, reconstitute the residue with 200μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 DeltaBond CN (Keystone Scientific, Bellafonte, PA)
Mobile phase: MeCN:MeOH:water 5:5:90 containing 10 mM pH 3 ammonium phosphate dibasic and 10 mM pH 3 tetramethylammonium hydroxide
Flow rate: 1
Injection volume: 100
Detector: UV 287

CHROMATOGRAM

Retention time: 6.5
Internal standard: BMY-46317 (9)
Limit of quantitation: 10 ng/mL

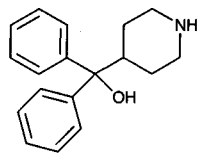
KEY WORDS

pharmacokinetics; radiolabeled; SPE; rat; plasma

REFERENCE

Marathe,P.H.; Greene,D.S.; Barbhaiya,R.H. Disposition of [14C]avitriptan in rats and humans, *Drug Metab.Dispos.*, **1997**, *25*, 881–888.

Azacyclonol



Molecular formula: $C_{18}H_{21}NO$
Molecular weight: 267.37
CAS Registry No.: 115-46-8, 1798-50-1 (HCl)
Merck Index: 925
Lednicer No.: 1 47

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

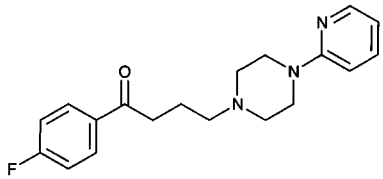
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Azaperone



Molecular formula: $C_{19}H_{22}FN_3O$
Molecular weight: 327.40
CAS Registry No.: 1649-18-9
Merck Index: 931
Lednicer No.: 2 300

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES

Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 1 ng/g

OTHER SUBSTANCES

Extracted: azaperol, carazolol, acepromazine, xylazine, haloperidol, propiomazine, chlorpromazine

KEY WORDS

SPE; pig; kidney

REFERENCE

Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the

eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES

Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 2 ng/g

OTHER SUBSTANCES

Extracted: azaperol, acepromazine, carazolol, xylazine, haloperidol, propriomazine, chlorpromazine

KEY WORDS

SPE; pig; kidney; liver

REFERENCE

Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

SAMPLE

Matrix: urine
Sample preparation: Adjust 75 mL urine to pH 10 with 10 mL saturated borate buffer and concentrated ammonium hydroxide, extract with 100 mL dichloromethane:isopropanol 96:4. Extract the organic layer with 100 mL 100 mM HCl, adjust the pH of the aqueous layer to 10, extract the aqueous layer with 100 mL hexane:isopropanol 96:4. Dry the extract at 50° under a stream of nitrogen, reconstitute the residue in 500 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 μm Suplex pKb-100 (Supelco)
Mobile phase: Gradient. MeCN:50 mM pH 10 ammonium acetate from 20:80 to 100:0 over 20 min.
Flow rate: 0.2
Detector: MS, Sciex API III triple quadrupole LC-MS-MS, heated nebulizer interface at 400°, corona discharge current 3 μA, orifice diameter 125 μm, collision induced dissociation using argon, argon curtain thickness was 500×10^{12} molecules/cm^2, collision energy 50 eV, positive ion mode

CHROMATOGRAM

Retention time: 20.1

OTHER SUBSTANCES

Extracted: metabolites

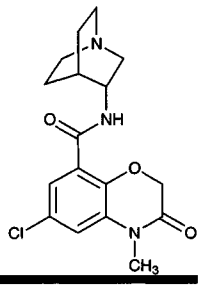
KEY WORDS

horse; LC-MS

REFERENCE

Chui,Y.C.; Esaw,B.; Laviolette,B. Investigation of the metabolism of azaperone in the horse, *J.Chromatogr.B*, **1994**, *652*, 23–33.

Azasetron



Molecular formula: $C_{17}H_{20}ClN_3O_3$
Molecular weight: 349.82
CAS Registry No.: 123040-69-7, 123040-16-4 (HCl)
Merck Index: 933

SAMPLE

Matrix: blood
Sample preparation: Add 20 μL 2 M NaOH and 2 mL dichloroethane to 200 μL serum. Shake for 10 min, centrifuge at 3000 rpm for 5 min. Remove a 1.6 mL portion of the upper organic layer, add 80 μL 100 mM HCl, and 1.5 mL hexane, centrifuge at 3000 rpm for 5 min. Remove the upper organic layer, inject a 50 μL aliquot of the lower layer.

HPLC VARIABLES

Column: 150 × 4 SenshuPak ODS-0151-N
Mobile phase: MeCN:THF:100 mM pH 5 ammonium acetate 9.8:6.2:84
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: F ex 318 em 382

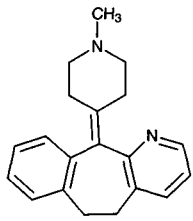
KEY WORDS

rabbit; serum; pharmacokinetics

REFERENCE

Moriyama,Y.; Arimori,K.; Nakano,M. Absorption characteristics of azasetron from rectal and oral routes in rabbits, *Biol.Pharm.Bull.*, **1997**, *20*, 701–703.

Azatadine



Molecular formula: $C_{20}H_{22}N_2$
Molecular weight: 290.41
CAS Registry No.: 3964-81-6, 3978-86-7 (maleate)
Merck Index: 934
Lednicer No.: 2 424

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.70 (A), 4.55 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 200 μL 5 μg/mL 8-chloroazatadine in water + 1 mL 1 M NaOH + 6 mL diethyl ether, shake in a reciprocal shaker for 10 min, centrifuge at 1600 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and add it to 500 μL 50 mM sulfuric acid, shake on a reciprocal shaker for 10 min, centrifuge at 1600 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Add the aqueous layer to 1 mL 1 M NaOH, extract with 6 mL hexane, centrifuge, freeze in dry ice/acetone. Remove the organic layer and add it to 100 μL concentrated ammonium hydroxide. Evaporate to dryness under a stream of nitrogen at 45°, dissolve the residue in 500 μL mobile phase, inject a 200 μL aliquot.

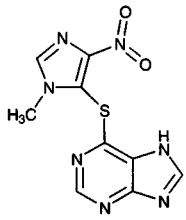
HPLC VARIABLES
Guard column: silica Guard-Pak (Waters)
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeCN:50 mM pH 7.5 KH_2PO_4 90:200
Flow rate: 2
Injection volume: 200
Detector: UV 214

CHROMATOGRAM
Retention time: 7.5
Internal standard: 8-chloroazatadine (10.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: chlorpheniramine, brompheniramine
Noninterfering: pseudoephedrine, phenylpropanolamine

REFERENCE
Alton,K.B.; Petruzzi,R.F.; Patrick,J.E. High-performance liquid chromatographic assay for azatadine in human urine, *J.Chromatogr.*, **1987**, *385*, 249–259.

Azathioprine



Molecular formula: $C_9H_7N_7O_2S$
Molecular weight: 277.27
CAS Registry No.: 446-86-6
Merck Index: 935
Lednicer No.: 2 464

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2.5 mL MeOH and 5 mL 0.2% acetic acid. Mix 1 mL plasma with 40 μL saturated EDTA solution, add to the SPE cartridge. Wash with 2 mL 0.2% acetic acid, centrifuge at 2200 g for 5 min to remove excess water, elute with 2 mL MeOH, evaporate to dryness under a stream of nitrogen at 37°. Reconstitute the residue with 200 μL mobile phase, vortex for 30 s, centrifuge at 11000 g for 5 min, inject an 80 μL aliquot of the supernatant. (Prepare saturated EDTA solution by vortexing 2.5 g disodium (?) EDTA in 25 mL water for 5 min.)

HPLC VARIABLES

Guard column: 12.5 × 4 Zorbax ODS
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. A was MeCN:1 mM triethylamine 0.8:99.2, adjusted to pH 3.2 with phosphoric acid. B was MeCN:1 mM triethylamine 20:80, adjusted to pH 3.2 with phosphoric acid. A:B 100:0 for 5 min, to 50:50 over 2.5 min, maintain at 50:50 for 11.5 min to 100:0 over 2 min, re-equilibrate at initial conditions for 9 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 340

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; plasma; pharmacokinetics

REFERENCE

Van Os,E.C.; McKinney,J.A.; Zins,B.J.; Mays,D.C.; Schriver,Z.H.; Sandborn,W.J.; Lipsky,J.J. Simultaneous determination of azathioprine and 6-mercaptopurine by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 147–154.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 25 ng 9-methylazathioprine + 4.5 mL ethyl acetate, vortex for 1 min, centrifuge for 1 min, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure at 35°, reconstitute the residue in 250 μL mobile phase, vortex for 10 s, sonicate for 10 min, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4.6 5 μm LiChrospher 100 RP 18
Column: 250 × 4.6 5 μm LiChrospher 60 Rp-select B

Mobile phase: MeCN:10 mM pH 2.3 potassium phosphate buffer 12:88 (Flush with MeCN: buffer 50:50 for 2 min after each run.)
Column temperature: 22
Flow rate: 1
Injection volume: 200
Detector: UV 285

CHROMATOGRAM

Retention time: 16
Internal standard: 9-methylazathioprine (Add 400 mg anhydrous potassium carbonate and 200 μL methyl iodide to a solution of 220 mg azathioprine in 7 mL DMF at 0-5°, stir under nitrogen at 24 h, add 14 mL water, neutralize with 1 M HCl and sodium bicarbonate solution, filter, wash the solid with water, dry under vacuum to give 9-methylazathioprine (mp 174-5°). Purify by precipitating from DMF solution with water.) (29)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Noninterfering: 6-mercaptopurine

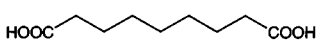
KEY WORDS

serum; pharmacokinetics

REFERENCE

Binscheck,T.; Meyer,H.; Wellhoner,H.H. High-performance liquid chromatographic assay for the measurement of azathioprine in human serum samples, *J.Chromatogr.B*, **1996**, *675*, 287–294.

Azelaic acid



Molecular formula: $C_9H_{16}O_4$
Molecular weight: 188.22
CAS Registry No.: 123-99-9
Merck Index: 938

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut NH2 SPE cartridge with 3 mL MeOH and 12 mL 100 mM pH 7 NaH_2PO_4 buffer. Add 1 mL plasma to the SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, dry under vacuum, elute with 1 mL 500 mM formic acid in MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 100 μL 50 mM triethylamine in MeCN, vortex for 1 min, add 50 μL 60 mM ethyl chloroformate in MeCN, vortex for 1 min, add 200 μL 3 mM L-leucine-(4-methyl-7-coumarinylamide) in MeOH, vortex for 1 min, let stand for 4 min, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL MeCN:water 50:50, inject a 5-15 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Axxiom octyl (Richard Scientific, Novato)
Mobile phase: Gradient. A was 1 L water containing 2 mL 85% phosphoric acid. B was 1 L MeCN containing 2 mL 85% phosphoric acid. A:B 55:45 for 15 min, to 10:90 over 15 min.
Injection volume: 5-15
Detector: F ex 330 em 390

CHROMATOGRAM

Retention time: 30

OTHER SUBSTANCES

Extracted: dodecanedioic acid, hexadecanedioic acid, pimelic acid, tetradecanedioic acid

KEY WORDS

plasma; derivatization; SPE; dog; human

REFERENCE

Levai,F.; Liu,C.-M.; Tse,M.M.; Lin,E.T. Pre-column fluorescence derivatization using leucine-coumarnylamide for HPLC determination of mono- and dicarboxylic acids in plasma, *Acta Physiol.Hung.*, **1995**, *83*, 39–46.

SAMPLE

Matrix: blood, feces, urine
Sample preparation: Serum. 1 mL Serum + 50 μg sebacic acid, acidify to pH 1 with 1 M HCl, saturate with NaCl, extract three times with 10 mL warm (40°) ethyl acetate. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to dryness under vacuum below 40°, take up the residue in 1 mL MeCN:MeOH 80:20, add 3 mg of p-bromophenacyl bromide in MeCN, add 6 μL N,N-diisopropylethylamine, heat at 50-60° for 10-15 min, evaporate some solvent, add reaction mixture to a TLC plate (Carlo Erba Stratocrom SI-AP, 0.25 mm silica gel, activated at 120° for 20 min), develop plate in benzene:hexane 3:1 (CAUTION! Benzene is a carcinogen!), remove material remaining at origin, extract three times with 1 mL MeCN, evaporate solvent to 500 μL, inject a 20-50 μL aliquot. Urine, feces. 20-50 μL Urine or feces diluted with water + 50 μg sebacic acid, acidify to pH 1 with 1 M HCl, extract five times with 3 volumes of warm (40°) ethyl acetate. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to

dryness under vacuum below 40°, take up the residue in 1 mL MeCN:MeOH 80:20, add 3 mg of p-bromophenacyl bromide in MeCN, add 6 μL N,N-diisopropylethylamine, heat at 50-60° for 10-15 min, evaporate some solvent, add reaction mixture to a TLC plate (Carlo Erba Stratocrom SI-AP, 0.25 mm silica gel, activated at 120° for 20 min), develop plate in benzene:hexane 3:1 (CAUTION! Benzene is a carcinogen!), remove material remaining at origin, extract three times with 1 mL MeCN, evaporate solvent to 500 μL, inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm RP 18 (Brownlee)
Mobile phase: Gradient. MeCN:water adjusted to pH 3.10 with phosphoric acid 60:40 for 5 min then to 100:0 over 60 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20-50
Detector: UV 255

CHROMATOGRAM
Retention time: 32.2
Internal standard: sebacic acid (35.9)
Limit of detection: 0.5 ng

OTHER SUBSTANCES
Extracted: other dicarboxylic acids (C4-C13)

KEY WORDS
serum; human; rat

REFERENCE
Passi,S.; Nazzaro-Porro,M.; Picardo,M.; Mingrone,G.; Fasella,P. Metabolism of straight saturated medium chain length (C9 to C12) dicarboxylic acids, *J.Lipid Res.*, **1983**, *24*, 1140–1147.

SAMPLE
Matrix: follicles, skin
Sample preparation: Wash skin with 500 μL acetone, homogenize follicles in acetone, centrifuge at 13400 g for 5 min, remove a 400 μL portion of the supernatant, re-centrifuge. Evaporate a 30 μL aliquot, take up the residue in 1 mL MeCN:MeOH 80:20, add 3 mg of p-bromophenacyl bromide in MeCN, add 6 μL N,N-diisopropylethylamine, heat at 50-60° for 10-15 min, evaporate some solvent, add reaction mixture to a TLC plate (Carlo Erba Stratocrom SI-AP, 0.25 mm silica gel, activated at 120° for 20 min), develop plate in benzene:hexane 3:1 (CAUTION! Benzene is a carcinogen!), remove material remaining at origin, extract three times with 1 mL MeCN, evaporate solvent to 50 μL, inject a 20-50 μL aliquot.

HPLC VARIABLES
Guard column: 24 × 3.9 Bondapak C18
Column: 150 × 3.9 Novapak C18
Mobile phase: Gradient. MeCN:water adjusted to pH 3.10 with phosphoric acid 60:40 for 5 min then to 100:0 over 60 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20-50
Detector: UV 254

CHROMATOGRAM
Limit of detection: 50 ng/mL

REFERENCE

Bojar,R.A.; Cutcliffe,A.G.; Graupe,K.; Cunliffe,W.J.; Holland,K.T. Follicular concentrations of azelaic acid after a single topical application, *Br.J.Dermatol.*, **1993**, *129*, 399–402.

SAMPLE

Matrix: formulations

Sample preparation: Condition a 1 g 100 μm Bakerbond Florisil SPE cartridge with 5 mL THF:hexane 40:60. Condition a 500 mg 40 μm Bakerbond C18 SPE cartridge with MeCN and MeCN:water 65:35. 200 mg Cream + 3 mL THF:hexane 40:60, sonicate, centrifuge at 3000 rpm for 2 min, repeat extraction twice. Add the supernatants to the Florisil SPE cartridge, wash with 2 mL THF:hexane 40:60, dry under vacuum, elute with two 2 mL portions of hexane:isopropanol 70:30. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 10 mL MeOH, neutralize (phenolphthalein endpoint) with 0.01% KOH in MeOH, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute with 15 mL MeCN, add 5 mL 2 mM p-bromophenacyl bromide in MeCN containing 100 μM 18-crown-6, add 10 mL MeCN, stir at 80° for 30 min, cool to 4°, dilute 1:50 with MeCN:water 65:35, add a 1 mL aliquot to the C18 SPE cartridge, wash with two 3 mL aliquots of MeCN:water 75:25, elute with 10 mL MeCN. Remove a 1 mL aliquot of the eluate and add it to 100 μL 85.125 μg/mL sebacic acid, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 100-RP-18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 6.06
Internal standard: sebacic acid (7.59)

KEY WORDS

derivatization; cream; SPE

REFERENCE

Ferioli,V.; Rustichelli,C.; Vezzalini,F.; Gamberini,G. Determination of azelaic acid in pharmaceuticals and cosmetics by RP-HPLC after pre-column derivatization, *Farmaco*, **1994**, *49*, 421–425.

SAMPLE

Matrix: formulations

Sample preparation: Ointment, lotion. Weigh out ointment or lotion equivalent to about 15 mg azelaic acid, dissolve in 100 mL MeOH, dilute an aliquot 1:5 with water. 200 μL Sample + 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, stir for 33 min at 70°, add 150 μL 20 μg/mL IS in MeCN, sonicate for 1 min, inject a 50 μL aliquot into mobile phase A. Ointment. Dissolve in MeCN to give a concentration of 18 μg/mL. 100-200 μL Sample + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN + 100 μL 1% triethylamine in MeCN, heat at 40° for 40 min, reconstitute in 150 μL 40 μg/mL IS in Mobile Phase B and 450 μL mobile phase B, sonicate for 1 min, inject a 50 μL aliquot into mobile phase B. Powder. Weigh out powder equivalent to about 15 mg azelaic acid, dissolve in 100 mL MeOH, sonicate for 10 min, centrifuge at 4000 rpm for 20 min, filter the supernatant, dilute an aliquot of the filtrate 1:5 with water. 200 μL Sample + 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, stir for 33 min at 70°, add 150 μL 20 μg/mL IS in MeCN, sonicate for 1 min, inject a 50 μL aliquot into mobile phase A. (Synthesis of 2-bromoacetyl-6-methoxynaphthalene is as follows. Stir

equimolar amounts of 2-acetyl-6-methoxynaphthalene (6'-methoxy-2'-acetonaphthone, Aldrich) and methyltriphenylphosphonium tribromide in anhydrous THF under nitrogen at room temperature for 1 h, dilute the reaction mixture with ether, wash with sodium bisulfite solution, wash with water (Phosphorus and Sulfur 1985, 25, 357). [Bromination can also be achieved with phenyltrimethylammonium tribromide over 3 h but the reaction is less selective.] Purify the crude product by column chromatography on silica gel using chloroform:petroleum ether 50:50 to give 2-bromoacetyl-6-methoxynaphthalene (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 Hypersil 5 ODS
Mobile phase: MeCN:MeOH:THF:water 38.5:28:3.5:30 (A) or 37.4:27.2:3.4:32
Column temperature: 35
Flow rate: 1.2 (A), 1.6 (B)
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 18
Internal standard: valproic acid 6-methoxynaphthacylester (15.5) [Prepare by dissolving 2 mmoles valproic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 0.5 mL triethylamine, heat at 60° for 30 min, cool, dilute with 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the extracts, wash with 5% sodium bicarbonate, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate under reduced pressure, recrystallize from MeOH/water to give white crystals, mp 56-7° (Chromatographia 1992, 33, 13).]

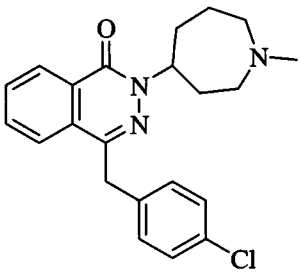
KEY WORDS

ointment; lotion; powder; derivatization

REFERENCE

Gatti,R.; Andrisano,V.; Di Pietra,A.M.; Cavrini,V. Analysis of aliphatic dicarboxylic acids in pharmaceuticals and cosmetics by liquid chromatography (HPLC) with fluorescence detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 589–595.

Azelastine



Molecular formula: $C_{22}H_{24}ClN_3O$
Molecular weight: 381.90
CAS Registry No.: 58581-89-8, 79307-93-0 (HCl)
Merck Index: 939
Lednicer No.: 4 152

SAMPLE

Matrix: blood, tissue
Sample preparation: 1 mL Plasma or tissue homogenate (equivalent to 100 mg tissue) + 100 μL 150 ng/mL IS + 250 μL 1 M NaOH + 9 mL hexane:octanol 95:5, extract. Add the organic layer to 125 μL 0.2% acetic acid, extract, centrifuge, inject 90 μL of the aqueous layer.

HPLC VARIABLES

Column: 250 mm long 5 μm Hypersil CPS
Mobile phase: MeCN:9 mM pH 3.0 triethylammonium phosphate (sic) 50:50 (plasma) or MeCN:water:triethylamine:phosphoric acid 500:500:0.4:0.2 (tissue)
Column temperature: 60 (tissue), 40 (plasma)
Flow rate: 0.45 (plasma), 0.60 (tissue)
Injection volume: 90
Detector: F ex 215 em 360

CHROMATOGRAM

Internal standard: 4-(p-chlorobenzyl)-2-[N-methyl-2,6-ethanopiperidinyl-(4)]-1-(2H)-phthalazinone hydrochloride (9.97 (plasma), 9.61 (tissue))
Limit of quantitation: 36 ng/g (tissue), 0.288 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; guinea pig; lung

REFERENCE

Adusumalli,V.E.; Wong,K.K.; Kucharczyk,N.; Sofia,R.D. Pharmacokinetics of azelastine and its active metabolite, desmethylazelastine, in guinea pigs, *Drug Metab.Dispos.*, **1992**, *20*, 530–535.

SAMPLE

Matrix: blood, tissue
Sample preparation: Tissue. Homogenize lung tissue in ten volumes water (Tissumizer). 1 g Homogenate + 100 μL 10 μg/mL IS in 0.2% acetic acid + 100 μL 10 M NaOH + 9 mL hexane:n-octanol 95:5, rotate for 1 h at 50 rpm, centrifuge. Remove 8 mL organic layer and add it to 125 μL 0.2% acetic acid, vortex vigorously, inject an aliquot of the aqueous layer. Plasma. 1 mL Plasma + 100 μL 150 ng/mL IS in 0.2% acetic acid + 250 μL 1 M NaOH + 9 mL hexane:n-octanol 95:5, rotate for 1 h at 50 rpm, centrifuge. Remove 8 mL organic layer and add it to 125 μL 0.2% acetic acid, vortex vigorously, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 2 5 μm Hypersil CPS
Mobile phase: MeCN:9 mM pH 3.0 triethylammonium phosphate (sic) 50:50 (plasma) or MeCN:water:triethylamine:phosphoric acid 500:500:0.4:0.2 (tissue)

Column temperature: 60 (tissue), 40 (plasma)
Flow rate: 0.45 (plasma), 0.60 (tissue)
Detector: F ex 215 em 360

CHROMATOGRAM
Retention time: 7.88 (plasma), 8.27 (tissue)
Internal standard: 4-(p-chlorobenzyl)-2-[N-methyl-2,6-ethanopiperidinyl-(4)]-1-(2H)-phthalazinone hydrochloride (9.97 (plasma), 9.61 (tissue))
Limit of quantitation: 0.039 ng/g (tissue), 0.156 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; guinea pig; lung

REFERENCE
Langevin,C.N.; Pivonka,J.; Wichmann,J.K.; Kucharczyk,N.; Sofia,R.D. High performance liquid chromatographic determination of azelastine and desmethylazelastine in guinea pig plasma and lung tissue, *Biomed.Chromatogr.*, **1993**, *7*, 7–11.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH and dilute with water to a concentration of 20 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 conalbumin-conjugated silica gel (React 2 g Unisil Q NH_2 (Macherey-Nagel) and 3 g N,N-disuccinimidylcarbonate in 50 μL MeCN for 6 h at room temperature, filter, wash silica gel with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Add silica gel to 2 g conalbumin (ovotransferrin) (from egg white) in 50 mL 50 mM pH 7.5 phosphate buffer, stir at room temperature for 6 h, filter, wash with water, wash with isopropanol: water 1:2, pack into columns.)
Mobile phase: EtOH:50 mM pH 5.0 potassium phosphate buffer 8:92
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 20 (d), 26 (l)

KEY WORDS
chiral

REFERENCE
Mano,N.; Oda,Y.; Miwa,T.; Asakawa,N.; Yoshida,Y.; Sato,T. Conalbumin-conjugated silica gel, a new chiral stationary phase for high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *603*, 105–109.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher Si-60
Column: 250 × 4 5 μm LiChrospher Si-60
Mobile phase: MeOH containing 0.033% perchloric acid

Flow rate: 0.5 for 17 min then 0.9
Injection volume: 20
Detector: F ex 210 em 360

CHROMATOGRAM

Retention time: 27
Internal standard: azelastine

OTHER SUBSTANCES

Simultaneous: flezelastine

KEY WORDS

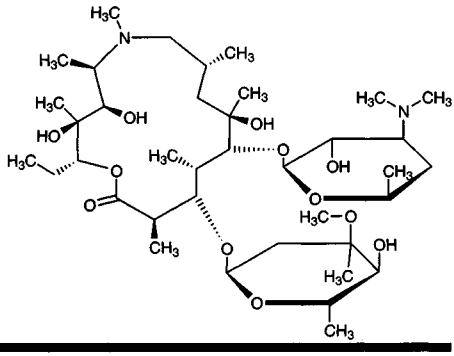
azelastine is IS

REFERENCE

Paris,S.; Blaschke,G.; Locher,M.; Borbe,H.O.; Engel,J. Investigation of the stereoselective in vitro metabolism of the chiral antiasthmatic/antiallergenic drug flezelastine by high-performance liquid chromatography and capillary zone electrophoresis, *J.Chromatogr.B*, **1997**, *691*, 463–471.

Azithromycin

Molecular formula: $C_{38}H_{72}N_2O_{12}$
Molecular weight: 749.00
CAS Registry No.: 83905-01-5
Merck Index: 946



SAMPLE

Matrix: blood
Sample preparation: Mix 200 μL plasma with 50 μL IS, add 200 μL 100 mM sodium carbonate, vortex for 30 s, add 3.5 mL MTBE, mix for 20 min, centrifuge at 2000 g for 10 min. Evaporate the MTBE layer to dryness at 37°, reconstitute the residue in 200 μL mobile phase, mix for 20 min, centrifuge, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 55:45
Flow rate: 1
Injection volume: 80
Detector: E, ESA Coulochem 5100 A, ESA 5010 dual electrode analytical cell at +680 mV and +780 mV, ESA 5020 guard cell +1.0 V

CHROMATOGRAM

Internal standard: clarithromycin
Limit of detection: 10 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Patel,K.B.; Xuan,D.; Tessier,P.R.; Russomanno,J.H.; Quintiliani,R.; Nightingale,C.H. Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2375–2379.

SAMPLE

Matrix: blood, bronchoalveolar lavage fluid
Sample preparation: Blood. Centrifuge 10 mL blood at 2500 g, separate the serum. Peripheral blood monocytes (PBM). Expose the PBM pellet to several freeze-thaw cycles, dilute the cell pellet in 1 mL saline with 1 mL serum and saline to volume 5 mL. Bronchoalveolar lavage (BAL) fluid. Expose the BAL pellet to several freeze-thaw cycles, dilute sample with water to volume 50 mL, freeze and thaw.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Chromegabond Alkylphenyl aluminum oxide (E.S. Industries, N.J.)
Mobile phase: MeCN:20 mM pH 10 Na_3PO_4 75:25
Flow rate: 1.5
Detector: E, Bioanalytical Systems, dual glassy carbon electrodes 600 and 850 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Limit of detection: 2 ng/mL (BAL and leucocytes), 10 ng/mL (serum)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Olsen,K.M.; San Pedro,G.S.; Gann,L.P.; Gubbins,P.O.; Halinski,D.M.; Campbell,G.D.,Jr. Intrapulmonary pharmacokinetics of azithromycin in healthy volunteers given five oral doses, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2582–2585.

SAMPLE
Matrix: blood, gastric juice, gastric mucosa, saliva, vitreous humor
Sample preparation: Homogenize 5-20 mg gastric mucosa in 300 μL 10 mM pH 7.4 sodium phosphate buffer with sonication. Add 500 ng roxithromycin in MeOH:water 50:50 to 500 μL plasma, serum, saliva, gastric juice, leucocytes lysate, vitreous humor, or 300 μL gastric mucosa homogenate, vortex, add 200 μL 100 mM sodium carbonate and 3 mL MTBE, shake thoroughly (5 × 2 s in an SMI Multi-tube vortexer), centrifuge at 1000 g for 5 min, freeze the aqueous layer in liquid nitrogen or in a freezer at -70° for 15 min. Evaporate the upper organic layer to dryness in a centrifugal vacuum evaporator (Jouan RC 10.22), reconstitute the residue in 250 μL MeOH:water 50:50, inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB CN
Mobile phase: MeCN:MeOH:50 mM Na_2HPO_4 and NaH_2PO_4 buffer 52.2:4.3:43.5 (pH 6.8) (The mobile phase was a mixture of 600 mL MeCN, 50 mL MeOH and 500 mL 50 mM Na_2HPO_4 and NaH_2PO_4 buffer.)
Column temperature: 30
Flow rate: 1
Injection volume: 20-50
Detector: E, ESA Coulochem II, guard cell +1.0 V, screening cell E1 +0.50 V, analytical cell E2 +0.80 V

CHROMATOGRAM
Retention time: 12
Internal standard: roxithromycin (5.5), clarithromycin (10)
Limit of detection: 10 ng/mL

KEY WORDS
gastric juice; pharmacokinetics; plasma; saliva; serum

REFERENCE
Kees,F.; Spangler,S.; Wellenhofer,M. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 287–293.

SAMPLE
Matrix: tears
Sample preparation: Add 10 μL 20 μ g/mL IS and 500 μL of 60 mM sodium carbonate to 50 μL tears. Extract with 5 mL of MTBE. Evaporate the organic layer to dryness under a stream of nitrogen at room temperature. Reconstitute the sample in 100 μL MeCN: water 50:50, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 4 μm Nova-Pak C18
Column: 100 × 8 4 μ m Nova-Pak C18 radial compression
Mobile phase: MeCN:MeOH:buffer 19:9:75 (Buffer was 35 mM Na_2HPO_4 containing 5 mM tetrabutylammonium phosphate and 5 mM sodium perchlorate, adjusted to pH 7.0 with phosphoric acid. Mobile phase was recycled and the buffer was prepared fresh every other day.)

Column temperature: 26
Flow rate: 4.0
Injection volume: 80
Detector: E, Coulochem 11, 5021 conditioning cell 0.7 V, 5011 analytical cell at 0.85 V

CHROMATOGRAM

Internal standard: n-propylazithromycin
Limit of quantitation: 100 ng/mL

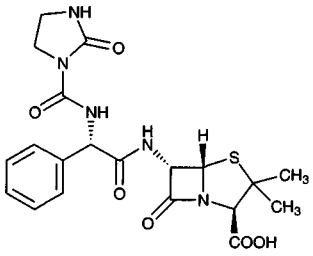
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Raines,D.A.; Yusuf,A.; el-Yazigi,A. Simultaneous analysis of azithromycin and two of its metabolites in human tears (Abstract 2501), *Pharm.Res.*, **1997**, *14*, S377–S377.

Azlocillin



Molecular formula: $C_{20}H_{23}N_5O_6S$
Molecular weight: 461.50
CAS Registry No.: 37091-66-0, 37091-65-9 (monosodium salt)
Merck Index: 947
Lednicer No.: 3 206

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10.

HPLC VARIABLES

Column: 75 × 4.6 3 μm octadecylsilane
Mobile phase: 20:80 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 5
Detector: UV 214

CHROMATOGRAM

Retention time: 2.2
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood, bronchial secretions
Sample preparation: Add an equal volume of digestor to the bronchial secretion, shake to fluidify sample. 1 mL Serum or diluted bronchial secretion + 1 mL MeCN, vortex, centrifuge at 5000 rpm for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex, centrifuge. Remove the aqueous supernatant and inject an aliquot.

HPLC VARIABLES

Column: endcapped 5 μm Lichrospher RP 18
Mobile phase: MeCN:pH 7 phosphate buffer 20:80
Flow rate: 0.8
Injection volume: 4.10
Detector: UV 220

CHROMATOGRAM
Retention time: 20

KEY WORDS
serum

REFERENCE
Condomines,M.; Mallet,M.N.; Albanese,J.; Gouin,F.; De Micco,P. A rapid high-performance liquid chromatography method for determining β-lactam antibiotics in biological fluids and tissues, *Chemioterapia*, **1987**, *6*, 251–253.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 1:20 with water. 200 μL Serum or diluted urine + 1 mL 20 μg/mL mezlocillin in MeCN, vortex, centrifuge, inject a 10-20 μL aliquot of the supernatant.

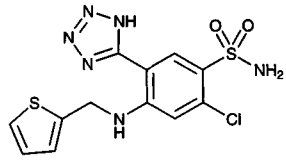
HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:67 mM pH 3.0 KH_2PO_4 45:55
Flow rate: 1
Injection volume: 10-20
Detector: UV 231.1

CHROMATOGRAM
Retention time: 10.1
Internal standard: mezlocillin (12.9)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Barriere,S.L.; Catlin,D.H.; Orlando,P.L.; Noe,A.; Frost,R.W. Alteration in the pharmacokinetic disposition of ciprofloxacin by simultaneous administration of azlocillin, *Antimicrob.Agents Chemother.*, **1990**, *34*, 823–826.

Azosemide



Molecular formula: $C_{12}H_{11}ClN_6O_2S_2$
Molecular weight: 370.84
CAS Registry No.: 27589-33-9
Merck Index: 953
Lednicer No.: 3 27

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Plasma, urine. 50 µL Plasma or urine + 125 µL MeCN, vortex, centrifuge at 9000 g for 10 min, inject 50 µL of the supernatant. Tissue. Homogenize with four volumes of 0.9% NaCl (Tissuemizer), centrifuge at 9000 g for 10 min. Remove 50 µL of the supernatant and add it to 125 µL MeCN, vortex, centrifuge at 9000 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 10 µm C18 (Waters)
Mobile phase: MeCN:30 mM phosphoric acid 40:50
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 6.0
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: furosemide, bumetanide, hydrochlorothiazide, amiloride, spironolactone

KEY WORDS

plasma; human; rabbit; rat; blood; liver; lung; heart; brain; kidney; muscle; stomach; intestine; spleen; pharmacokinetics

REFERENCE

Lee,S.H.; Lee,M.G. Determination of azosemide and its metabolite in plasma, blood, urine and tissue homogenates by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 367–372.

SAMPLE

Matrix: blood, urine
Sample preparation: Urine. Filter (0.45 µm). Remove a 300 µL aliquot and add it to 300 µL water and 50 µL 412 µg/mL phenobarbital in EtOH, vortex, inject a 10 µL aliquot. Serum. 300 µL (?) Serum + 20 µL 412 µg/mL phenobarbital in EtOH + 400 µL MeCN, mix, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50-100 µL buffer, inject a 5-20 µL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 CO:Pell ODS (Whatman)
Column: 250 × 4.6 5 µm Zorbax ODS C18
Mobile phase: Gradient. MeCN:buffer from 10:90 to 40:60 over 10 min, maintain at 40:60 for 2 min, re-equilibrate for 5 min. (Buffer was 0.6 mL glacial acetic acid in 1 L water, adjust pH to 4.05 with 4 M NaOH.)
Flow rate: 2
Injection volume: 5-20

Detector: UV 239

CHROMATOGRAM
Retention time: 10
Internal standard: phenobarbital (11)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, aspirin, chlorothiazide, chlorpromazine, hydrochlorothiazide, procainamide, quinidine, sulfamethoxazole, theophylline, tolbutamide

KEY WORDS
serum; pharmacokinetics

REFERENCE
Seiwell,R.; Brater,C. Separation and analysis of azosemide in urine and in serum by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 257–261.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 250 μL MeCN, vortex, centrifuge, inject an aliquot of the supernatant.

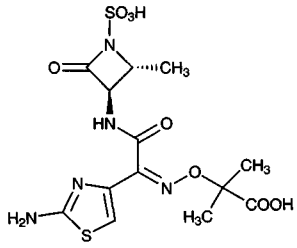
HPLC VARIABLES
Column: 300 × 3.9 10 μm C18 (Waters)
Mobile phase: MeCN:30 mM phosphoric acid 40:50
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: 6
Limit of detection: 90 ng/mL

KEY WORDS
rabbit; plasma; pharmacokinetics

REFERENCE
Lee,S.H.; Shin,W.G.; Lee,M.G.; Kim,N.D. Arterial and venous blood sampling in pharmacokinetic studies: azosemide in rabbits, *Biopharm.Drug Dispos.*, **1994**, *15*, 305–316.

Aztreonam



Molecular formula: $C_{13}H_{17}N_5O_8S_2$
Molecular weight: 435.44
CAS Registry No.: 78110-38-0
Merck Index: 955
Lednicer No.: 4 193, 195

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 33:67 MeCN:10 mM ammonium acetate + 5 mM tetrabutylammonium bromide adjusted to pH 7 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.2
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Dilute serum with an equal volume of MeCN, centrifuge at 15000 g for 2 min, inject a 50-200 μL aliquot of the supernatant. Urine. Dilute urine tenfold with 5 mM pH 3.0 tetrabutylammonium hydrogen sulfate, inject a 50-200 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 3.9 Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: Human serum, human urine. MeCN:5 mM tetrabutylammonium hydrogen sulfate and 5 mM ammonium sulfate adjusted to pH 3.0 with 1 M K_2HPO_4 20:80; Mouse serum, mouse urine, monkey urine. MeCN:buffer 20:80; Rat serum, monkey serum. MeCN:buffer 35:65; Rat urine, rabbit urine. MeCN:buffer 17.5:82.5; Rabbit serum. MeCN:

buffer 25:75 (Buffer was 5 mM tetrabutylammonium hydrogen sulfate adjusted to pH 3.0 with 1 M K_2HPO_4.)
Flow rate: 2
Injection volume: 50-200
Detector: UV 293

CHROMATOGRAM
Retention time: 5 (human serum and urine)
Limit of detection: 5000 ng/mL (urine), 1000 ng/mL (serum)

KEY WORDS
serum; human; monkey; rat; mouse; rabbit

REFERENCE
Pilkiewicz,F.G.; Remsburg,B.J.; Fisher,S.M.; Sykes,R.B. High-pressure liquid chromatographic analysis of aztreonam in sera and urine, *Antimicrob.Agents Chemother.*, **1983**, *23*, 852–856.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Solution + 4.9 mL MeOH:water 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Adsorbosphere C18
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:5 mM pH 2.6 ammonium phosphate containing 2 mM tetrabutylammonium hydroxide 26:74
Flow rate: 1
Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 14.0

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Inagaki,K.; Gill,M.A.; Okamoto,M.P.; Takagi,J. Stability of ranitidine hydrochloride with aztreonam, ceftazidime, or piperacillin sodium during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1992**, *49*, 2769–2772.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1: 8 with water, combine a 100 μL aliquot of the diluted solution with 100 μL cimetidine solution and 200 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 3.9 × 300 μBondapak C18
Mobile phase: 5 mM tetrabutylammonium hydrogen sulfate, 7% MeCN, 14% MeOH in 10 mM phosphate buffer (pH 2.6-2.7)
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 7.05
Internal standard: cimetidine (3.27)

OTHER SUBSTANCES
Simultaneous: with ampicillin, sulbactam

KEY WORDS
injections; stability-indicating; saline

REFERENCE
Belliveau,P.P.; Nightingale,C.H.; Quintiliani,R. Stability of aztreonam and ampicillin sodium-sulbactam sodium in 0.9% sodium chloride injection, *Am.J.Hosp.Pharm.*, **1994**, *51*, 901–904.

SAMPLE
Matrix: formulations
Sample preparation: Add cefazolin sodium (20 mg/mL), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 3 with 1 M KH_2PO_4.)
Flow rate: 2
Injection volume: 10
Detector: UV 293

CHROMATOGRAM
Retention time: 7.5
Internal standard: cefazolin (13)

KEY WORDS
injections; stability-indicating; 5% dextrose

REFERENCE
Bosso,J.A.; Prince,R.A.; Fox,J.L. Compatibility of ondansetron hydrochloride with fluconazole, ceftazidime, aztreonam, and cefazolin sodium under simulated Y-site conditions, *Am.J.Hosp.Pharm.*, **1994**, *51*, 389–391.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm C8 (Vydac)
Column: 250 × 4.6 5 μm C8 (Vydac)
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 15
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: vancomycin

KEY WORDS
stability-indicating; injections; 5% dextrose; saline

REFERENCE
Trissel,L.A.; Xu,Q.A.; Martinez,J.F. Compatibility and stability of aztreonam and vancomycin hydrochloride, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2560–2564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 6μm Zorbax CN
Mobile phase: 1.8 mM pH 4.1 copper sulphate
Flow rate: 0.7
Injection volume: 25
Detector: UV 232

CHROMATOGRAM
Retention time: 10.3
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: arginine

REFERENCE
Khedr,A. High-performance liquid chromatography of α-amino acids and aztreonam on reversed phase columns with aqueous Cu2+ as eluent, *Biomed.Chromatogr.*, **1996**, *10*, 167–171.

SAMPLE
Matrix: solutions
Sample preparation: Add 20 μL solution to 2 mL 1 mg/mL cefoperazone in water, vortex for 15 s, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C8
Mobile phase: MeCN:buffer 23:77 containing 1.7 g/L tetrabutylammonium hydrogen sulfate, pH adjusted to 3.5 with 5 M NaOH (The buffer was 60 mL 100 mM sodium acetate and 710 mL 100 mM acetic acid.)
Flow rate: 2
Injection volume: 10
Detector: UV 254

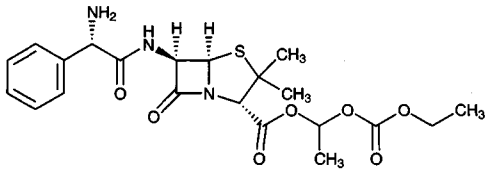
CHROMATOGRAM
Retention time: 8.0
Internal standard: cefoperazone (13.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
5% dextrose; saline

REFERENCE
Marble,D.A.; Bosso,J.A.; Townsend,R.J. Compatibility of clindamycin phosphate with aztreonam in polypropylene syringes and with cefoperazone sodium, cefonicid sodium, and cefuroxime sodium in partial-fill glass bottles, *Drug Intell.Clin.Pharm.*, **1988**, *22*, 54–57.

Bacampicillin



Molecular formula: $C_{21}H_{27}N_3O_7S$
Molecular weight: 465.53
CAS Registry No.: 50972-17-3, 37661-08-8 (HCl)
Merck Index: 961
Lednicer No.: 3 204

SAMPLE

Matrix: blood
Sample preparation: Add 100000 fold excess of ampicillin to eliminate adsorption of bacampicillin, extract into butyl acetate, re-extract into pH 2 buffer. Wash the aqueous phase with n-hexane, inject an aliquot.

HPLC VARIABLES

Column: 100 × 2.9 5 μm Nucleosil C18
Mobile phase: MeCN:pH 7.4 phosphate buffer 41:49
Injection volume: 20
Detector: F with post-column reaction. The column effluent mixed with two volumes of sodium borate buffer then with one volume of 150 μg/mL fluorescamine in acetone (Science 1972, 178, 871).

CHROMATOGRAM

Limit of detection: 800 pg/mL

REFERENCE

Sjövall,J.; Westerlund,D.; Alván,G.; Magni,L.; Nord,C.E.; Sörstad,J. Rectal bioavailability of bacampicillin hydrochloride in man as determined by reversed-phase liquid chromatography, *Chemotherapy*, **1984**, *30*, 137–147.

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma + 1 mL MeOH, stir for 5 min, centrifuge at 2400 g for 10 min. Remove 1 mL supernatant, add 2 μg cefazolin, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:67 mM KH_2PO_4 20:80
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 9 (measured as ampicillin peak)
Internal standard: cefazolin (14)
Limit of detection: 500 ng/mL

KEY WORDS

plasma

REFERENCE

Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E.; Picari,M. High-performance liquid chromatographic assay of ampicillin and its prodrug lenampicillin, *J.Chromatogr.*, **1990**, *507*, 235–239.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.687

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

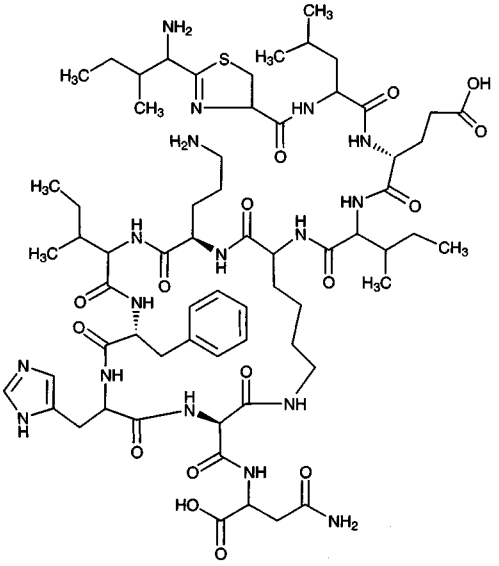
Bacitracin

Molecular formula: $C_{66}H_{103}N_{17}O_{16}S$ (bacitracin A)

Molecular weight: 1422.61 (bacitracin A)

CAS Registry No.: 1405-87-4, 1405-89-6 (zinc salt), 1405-88-5 (methylenedisalicylate)

Merck Index: 965



SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1 g in 100 mL 20 mM HCl in MeOH:water 80:20, mix, vortex, centrifuge, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC basic C8 200 Å (YMC)
Mobile phase: Gradient. MeOH:50 mM pH 6.5 KH_2PO_4 from 57:43 to 63:37 over 1 h
Column temperature: 25
Flow rate: 1
Injection volume: 100
Detector: UV 215

CHROMATOGRAM

Retention time: 14, 16, 20, 21, 25, 27, 30 (bioactive fractions)

REFERENCE

Bell,R.G. Preparative high-performance liquid chromatographic separation and isolation of bacitracin components and their relationship to microbiological activity, *J.Chromatogr.*, **1992**, *590*, 163–168.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in saline at a concentration of 1-10 mg/mL, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 C18 (Vydac)
Mobile phase: Gradient. MeCN:water (both containing 0.05% trifluoroacetic acid) from 5:95 to 65:35 over 20 min
Flow rate: 2
Detector: UV 210

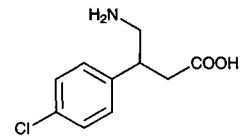
KEY WORDS

saline

REFERENCE

Drapeau,G.; Petitclerc,E.; Toulouse,A.; Marceau,F. Dissociation of the antimicrobial activity of bacitracin USP from its renovascular effects, *Antimicrob.Agents Chemother.*, **1992**, *36*, 955–961.

Baclofen



Molecular formula: $C_{10}H_{12}ClNO_2$
Molecular weight: 213.66
CAS Registry No.: 1134-47-0
Merck Index: 967
Lednicer No.: 2 121

SAMPLE

Matrix: CSF

Sample preparation: Prepare a 100 × 7 column of Dowex 50X4-400, treat with an excess of aqueous ammonia, wash with water until the washings are neutral, wash with an excess of 4 M HCl, wash with water until the washings are neutral, rinse with 8 mL water. Add 200 μL CSF to the column, wash with 8 mL water, elute with 2 mL 10% ammonia. Lyophilize the eluate, reconstitute the residue with 1 mL pH 7.4 phosphate buffer, extract twice with 1 mL portions of 1-butanol. Combine the organic layers and evaporate them to dryness, add 10-50 μL EtOH:water:triethylamine 50:25:25, evaporate to dryness, add 20 μL reagent, let stand at room temperature for 20 min, evaporate to dryness, reconstitute with pH 7.4 sodium phosphate buffer, inject a 5-25 μL aliquot. (Reagent was EtOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, store at -20° (Anal.Biochem. 1989, 176, 269).)

HPLC VARIABLES

Column: 150 × 3.9 Pico-Tag
Mobile phase: Gradient. A was sodium acetate adjusted to pH 6.4 with glacial acetic acid. B was MeCN:water 60:40. A:B from 100:0 to 60:40 over 10 min, to 0:100 over 1 min, maintain at 0:100 over 3 min.
Column temperature: 38
Injection volume: 5-25
Detector: UV 254

CHROMATOGRAM

Retention time: 13.2
Limit of detection: 5-10 ng/mL

KEY WORDS

derivatization; SPE; pharmacokinetics

REFERENCE

Sallerin-Caute,B.; Monsarrat,B.; Lazorthes,Y.; Cros,J.; Bastide,R. A sensitive method for the determination of baclofen in human CSF by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1988**, *11*, 1753–1761.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut SCX strong cation exchange SPE cartridge with 2 mL hexane, 2 mL MeOH, 2 mL water, and 3 mL saturated NaCl solution. 1 mL Plasma + 100 μL water + 1 mL citrate buffer, mix, add to the SPE cartridge, wash with 4 mL water, wash with 1 mL saturated NaCl solution, dry the SPE cartridge, elute with 1.5 mL pH 10.4 borate buffer. Mix a 200 μL aliquot of the eluate and add it to 50 μL reagent, mix, inject a 20 μL aliquot. (Prepare citrate buffer by diluting 89 mL 100 mM citric acid to 100 mL with 200 mM Na_2HPO_4, pH 2.6. Prepare pH 10.4 borate buffer by mixing 54 mL 200 mM boric acid in 100 mM NaOH with 46 mL 100 mM NaOH. Prepare pH 9.3 borate buffer by mixing 87 mL 200 mM boric acid in 100 mM NaOH with

13 mL 100 mM NaOH. Prepare reagent daily by mixing 75 mg o-phthalaldehyde, 5 mL MeOH, 50 μL tert-butanethiol, and 5 mL pH 9.3 borate buffer.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapak
Mobile phase: MeOH:buffer 74:36 (Prepare buffer by adjusting the pH of 60 mM Na_2HPO_4 to 7 with 60 mM KH_2PO_4.)
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5020 guard cell +1.2 V, Model 5011 glassy carbon working cell, screen electrode +0.2 V, quantifying electrode +0.7 V

CHROMATOGRAM
Retention time: 26
Limit of detection: 2.5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
derivatization; plasma; SPE; pharmacokinetics

REFERENCE
Millerioux,L.; Brault,M.; Gualano,V.; Mignot,A. High-performance liquid chromatographic determination of baclofen in human plasma, *J.Chromatogr.A*, **1996**, *729*, 309–314.

SAMPLE
Matrix: blood, CSF, dialysate, tissue
Sample preparation: Dilute dialysate and CSF. Homogenize brain tissue with 4 volumes of MeOH:water in an ice bath, let stand at -20° for 8 h. 30 μL Plasma + 60 μL MeOH, mix, let stand at -20° for 1 h, centrifuge at 4° at 10000 rpm for 5 min, dilute the supernatant with water. Mix an aliquot of tissue homogenate, deproteinized plasma, diluted dialysate, or diluted CSF with an equal volume of the reagent, let stand for 1.5 min, inject a 30 μL aliquot. (Prepare reagent by mixing 50 mg o-phthalaldehyde, 900 μL MeOH, 100 μL 400 mM pH 9.2 borate buffer, and 50 μL 2-mercaptoethanol.)

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 250 × 4.6 5 μm Finepak SIL C18S ODS (Jasco)
Mobile phase: MeOH:THF:100 mM pH 6.95 acetate buffer 45.5:2:52.5 (dialysate, CSF, plasma) or 43:2:55 (tissue)
Flow rate: 1
Injection volume: 30
Detector: F ex 368 em 434

CHROMATOGRAM
Limit of detection: 100 nM

KEY WORDS
plasma; derivatization; rat; brain; pharmacokinetics

REFERENCE
Deguchi,Y.; Inabe,K.; Tomiyasu,K.; Nozawa,K.; Yamada,S.; Kimura,R. Study on brain interstitial fluid distribution and blood-brain barrier transport of baclofen in rats by microdialysis, *Pharm.Res.*, **1995**, *12*, 1838–1844.

SAMPLE
Matrix: blood, urine

Sample preparation: 500 μL Plasma + 2 mL MeOH, centrifuge. 1 mL Supernatant or 100 μL urine + 500 μL pH 9 sodium tetraborate buffer + 250 μL 0.2% 4-chloro-7-nitrobenzofurazan in MeOH, heat at 60° for 45 min, acidify with 100 mM HCl, extract with 5 mL ethyl acetate. Evaporate 3 mL of the extract to about 500 μL, dry with anhydrous sodium sulfate, pass through SPE column, elute with ethyl acetate to give a final volume of 2 mL. Evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 10 μL aliquot. (Prepare the 25 × 6 SPE column with 0.063-0.200 mm silica gel (Merck), wet it with ethyl acetate.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm Bondapak C18
Mobile phase: MeOH:water 45:55
Flow rate: 0.4
Injection volume: 10
Detector: F ex 463 em 524

CHROMATOGRAM
Retention time: 4.0
Limit of detection: 100 ng/mL (urine), 20 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics; SPE; derivatization

REFERENCE
Tosunoglu,S.; Ersoy,L. Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection, *Analyst*, **1995**, *120*, 373–375.

SAMPLE
Matrix: formulations
Sample preparation: Dilute an aliquot with water to a concentration of 5 μg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Speri-5 ODS (Applied Biosystems)
Mobile phase: MeCN:50 mM NaH_2PO_4 20:80, adjusted to pH 3.5 with 85% phosphoric acid
Flow rate: 1
Injection volume: 15
Detector: UV 220

CHROMATOGRAM
Retention time: 5.5

KEY WORDS
syrup; stability-indicating

REFERENCE
Johnson,C.E.; Hart,S.M. Stability of an extemporaneously compounded baclofen oral liquid, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2353–2355.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:10 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 35-60 μm Perisorb RP18
Column: 250 × 4 10 μm LiChrosorb RP18
Mobile phase: MeOH:MeCN:2.72 g/L KH_2PO_4 2:12:86

Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 10.3

KEY WORDS
injections; water

REFERENCE
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 5 μm Adsorbosphere C18
Mobile phase: MeOH:buffer 40:60 (Buffer was 50 mM sodium acetate adjusted to pH 6.4 with glacial acetic acid.)
Flow rate: 1.0
Injection volume: 100
Detector: UV 266

CHROMATOGRAM
Limit of quantitation: 10 μg/mL

REFERENCE
A simple, rapid and reliable HPLC method for the analysis of baclofen in tablets (Abstract 3350), *Pharm.Res.*, **1997**, *14*, S581–S581.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer: 25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 5:95. B was MeCN:MeOH:50 mM pH 6.8 potassium phosphate buffer 55:10:35. A:B from 70:30 to 0:100 over 1 h, maintain at 0:100 for 20 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Simultaneous: amphetamine, tranylcypromine

KEY WORDS
derivatization

REFERENCE

Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE

Matrix: solutions

Sample preparation: 200 µL 10 mM Baclofen in 100 mM sodium bicarbonate + 200 µL 10 mM 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone (freshly prepared), stir at 40° for 1 h, cool, add 100 µL 200 mM HCl, inject an aliquot. (Derivatization in Anal.Biochem. 1992, 202, 210.)

HPLC VARIABLES

Column: 150 × 4.6 5 µm YMC.GEL C8 (YMC)
Mobile phase: MeOH:5% KH_2PO_4 (pH 4.0) 11:8
Flow rate: 1
Detector: UV 340

CHROMATOGRAM

Retention time: 17 (R-(-)), 20 (S-(+))

KEY WORDS

chiral; derivatization

REFERENCE

Shimada,K.; Mitamura,K.; Morita,M.; Hirakata,K. Separation of the diastereomers of baclofen by high performance liquid chromatography using cyclodextrin as a mobile phase additive, *J.Liq. Chromatogr.*, **1993**, *16*, 3311–3320.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4 5 µm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.71 (A), 3.47 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, fu-

rosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

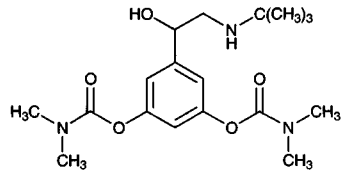
CHROMATOGRAM
Retention time: 9.32, 14.23

OTHER SUBSTANCES
Simultaneous: levodopa, norephedrine, primaquine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Bambuterol



Molecular formula: $C_{18}H_{29}N_3O_5$
Molecular weight: 367.45
CAS Registry No.: 81732-65-2, 81732-46-9 (HCl)
Merck Index: 980

SAMPLE

Matrix: blood
Sample preparation: Dilute plasma with buffer containing IS, inject an aliquot corresponding to 0.5 μL plasma.

HPLC VARIABLES

Guard column: 4 × 4 LiChrosorb RP-select B
Column: 50 × 4 5 μm LiChrospher RP-select B
Mobile phase: Gradient. MeOH:100 mM pH 5 ammonium acetate from 3:97 to 10:90 over 5 min, to 25:75 over 1 min, to 38:62 over 7 min
Flow rate: 1.4
Detector: MS, thermospray, Finnigan 4500 quadrupole, ion source repeller equipped with polyimide sleeve, trap at -90°, analyzer pressure 0.000035 Torr, manifold fore pressure 0.20 Torr, exhaust line pressure 1.1 Torr, repeller 45 V, vaporizer 115°, jet 200°, aerosol 270

CHROMATOGRAM

Retention time: 11
Internal standard: hedxadeuterobambuterol

KEY WORDS

plasma; LC-MS; dog

REFERENCE

Lindberg,C.; Paulson,J.; Blomqvist,A. Evaluation of an automated thermospray liquid chromatography-mass spectrometry system for quantitative use in bioanalytical chemistry, *J.Chromatogr.*, **1991**, *554*, 215–226.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with three 3 mL portions of EtOH, two 3 mL portions of water, and with 3 mL 10 mM pH 7.5 phosphate buffer. Add the incubation mixture to the SPE cartridge, wash with two 3 mL portions of water, elute with two 1 mL portions of EtOH:50 mM pH 8.5 ammonium chloride buffer 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 500 μL of the initial mobile phase, inject a 200 μL aliquot.; SPE

HPLC VARIABLES

Column: 150 × 5 Nucleosil 10SA
Mobile phase: Gradient. A was 250 mM pH 4.6 ammonium acetate buffer. B was MeCN: 500 mM pH 4.6 ammonium acetate buffer 50:50. From A:B 90:10 to 10:90 over 20 min.
Flow rate: 1
Injection volume: 200
Detector: UV 254

CHROMATOGRAM

Retention time: 16.5

OTHER SUBSTANCES
Extracted: terbutaline, metabolites

KEY WORDS
rat; SPE

REFERENCE
Lindberg,C.; Roos,C.; Tunek,A.; Svensson,L.Å. Metabolism of bambuterol in rat liver microsomes: identification of hydroxylated and demethylated products by liquid chromatography mass spectrometry, *Drug Metab.Dispos.*, **1989**, *17*, 311–322.

SAMPLE
Matrix: perfusate, tissue
Sample preparation: Perfusate. 400 μL Lung perfusate + 400 μL 5% perchloric acid, mix, centrifuge, inject a 200 μL aliquot of the supernatant. Tissue. Homogenize lung in 2 volumes of water (Polytron Homogenizer), mix 400 μL homogenate with 400 μL 5% perchloric acid, mix, centrifuge, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 5 Nucleosil 10SA
Mobile phase: Gradient. A was 19.3 g ammonium acetate and 14.4 mL acetic acid in 1 L water. B was 19.3 g ammonium acetate and 14.4 mL acetic acid in 1 L MeCN:water 50:50. A:B from 90:10 to 10:90 over 20 min, stay at 10:90 for 3 min, return to initial conditions over 3 min, re-equilibrate for 7 min.
Flow rate: 1
Injection volume: 200
Detector: UV 254

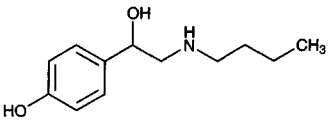
CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: metabolites, terbutaline

KEY WORDS
lung; guinea pig

REFERENCE
Ryrfeldt,Å.; Nilsson,E.; Tunek,A.; Svensson,L.-Å. Bambuterol: uptake and metabolism in guinea pig isolated lungs, *Pharm.Res.*, **1988**, *5*, 151–155.

Bamethan



Molecular formula: $C_{12}H_{19}NO_2$
Molecular weight: 209.29
CAS Registry No.: 3703-79-5, 5716-20-1 (sulfate)
Merck Index: 981
Lednicer No.: 2 39

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 5.91

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

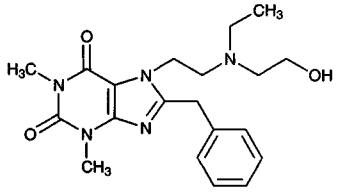
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Bamifylline



Molecular formula: $C_{20}H_{27}N_5O_3$
Molecular weight: 385.47
CAS Registry No.: 2016-63-9, 20684-06-4 (HCl)
Merck Index: 982
Lednicer No.: 1 426

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 10.288

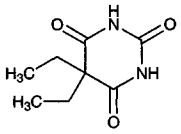
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Barbital



Molecular formula: $C_8H_{12}N_2O_3$
Molecular weight: 184.19
CAS Registry No.: 57-44-3, 144-02-5 (sodium salt)
Merck Index: 989
Lednicer No.: 1 267

SAMPLE

Matrix: blood
Sample preparation: Inject a 5-20 μL aliquot of serum directly onto the column with mobile phase A or B.

HPLC VARIABLES

Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 8:92 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B)
Flow rate: 1
Injection volume: 5 (A), 20 (B)
Detector: UV 254

CHROMATOGRAM

Retention time: 7.60 (A), 8 (B)

OTHER SUBSTANCES

Simultaneous: acetaminophen (B), carbamazepine (B), phenobarbital (B), phenytoin (B), primidone (B), sulfapyridine (B)

KEY WORDS

serum

REFERENCE

Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.445

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

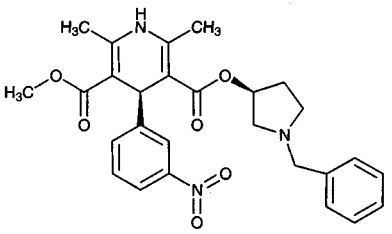
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, na-

lorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Barnidipine



Molecular formula: $C_{27}H_{29}N_3O_6$
Molecular weight: 491.54
CAS Registry No.: 104713-75-9
Merck Index: 1031

SAMPLE

Matrix: bile, urine
Sample preparation: Inject urine and bile directly. Hydrolyse urine or bile by heating a 1 mL aliquot with 200 µL 200 mM pH 5.5 sodium acetate buffer and 20 µL 100 U/mL β-glucuronidase at 37° for 24 h, add to a Sep-Pak C18 SPE cartridge, elute with MeOH. Evaporate the eluate, reconstitute the residue with MeOH:50 mM pH 5.0 sodium acetate buffer 5:95, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: Gradient. MeOH:50 mM pH 5.0 sodium acetate buffer 5:95 for 5 min, to 50:50 over 95 min
Flow rate: 0.8
Detector: UV

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; dog; SPE

REFERENCE

Teramura,T.; Tokunaga,T.; Matsumoto,H.; Watanabe,T.; Higuchi,S. Metabolism of barnidipine hydrochloride, a potent calcium antagonist, in rat and dog, *Xenobiotica*, **1996**, *26*, 177–187.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum or plasma + 500 µL 500 mM pH 11 phosphate buffer + 3 mL benzene (Caution! Benzene is a carcinogen!), shake mechanically for 5 min, centrifuge at 3000 rpm for 10 min. Remove 2.7 mL of the organic layer and add it to 50 ng IS, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 75 µL mobile phase, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm Aluspher RP-select B (Merck)
Mobile phase: MeCN:100 mM pH 11.8 Britton-Robinson buffer 40:60
Flow rate: 0.9
Injection volume: 5
Detector: E, JASCO Model 840-EC, glassy carbon working electrode 0.6 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.5
Internal standard: 2-(N-benzyl-N-methylamino) ethylmethyl 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate hydrochloride (YC-204, Yamanouchi) (7.3)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Noninterfering: acetazolamide, allopurinol, amitriptyline, chlorpromazine, clonidine, cyproheptadine, hydrochlorothiazide, imipramine, methyldopa, phenoxybenzamine, propranolol, ascorbic acid

KEY WORDS
human; dog; serum; plasma; protect from light; pharmacokinetics

REFERENCE
Takamura,K.; Abdel-Wadood,H.M.; Kusu,F.; Rafaat,I.H.; Saleh,G.A.; El-Rabbat,N.A.; Otagiri,M. Determination of barnidipine in human serum and dog plasma by HPLC with electrochemical detection, *Biol.Pharm.Bull.*, **1995**, *18*, 1311–1314.

SAMPLE
Matrix: blood, enzyme incubations
Sample preparation: 1 mL Enzyme incubation or plasma + 5 mL diethyl ether + 500 μL saturated sodium bicarbonate, extract, centrifuge at 800 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeOH:20 mM pH 3.0 bromo-tetra-n-propylammonium phosphate 50:50
Flow rate: 1
Detector: UV

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog; plasma

REFERENCE
Teramura,T.; Tokunaga,T.; Matsumoto,H.; Watanabe,T.; Higuchi,S. Metabolism of barnidipine hydrochloride, a potent calcium antagonist, in rat and dog, *Xenobiotica*, **1996**, *26*, 177–187.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL ice-cold MeOH, vortex, centrifuge at 1000 g for 15 min, filter (0.22 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: Gradient. MeCN:20 mM pH 7.0 ammonium acetate buffer 30:70 for 5 min, to 70:30 over 55 min, maintain at 70:30 for 30 min.
Flow rate: 1
Detector: radioactivity

CHROMATOGRAM
Retention time: 66

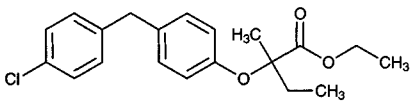
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog; liver; ^{14}C labeled

REFERENCE

Teramura,T.; Tokunaga,T.; Matsumoto,H.; Watanabe,T.; Higuchi,S. Metabolism of barnidipine hydrochloride, a potent calcium antagonist, in rat and dog, *Xenobiotica*, **1996**, *26*, 177–187.

Beclobrate



Molecular formula: $C_{20}H_{23}ClO_3$
Molecular weight: 346.85
CAS Registry No.: 55937-99-0
Merck Index: 1046

SAMPLE

Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL acetic acid + 1.75 mL 10 μg/mL IS in hexane, shake for 5 min, centrifuge at 2000 rpm for 20 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 30 × 4 30-40 μm RP18 (E. Merck)
Column: 125 × 4 5 μm LiChrosorb RP18
Mobile phase: MeCN:MeOH:water:acetic acid 40:30:30:0.1
Flow rate: 2
Injection volume: 50
Detector: UV 27

CHROMATOGRAM

Retention time: 4.1 (beclobric acid)
Internal standard: SGD 2774 (5.8)

KEY WORDS

plasma; pharmacokinetics; bioequivalence

REFERENCE

Gikalov,I.; Ifflaender,U. Pharmacokinetik und Bioäquivalenz von zwei peroralen Beclobrat-Zubereitungen [Pharmacokinetics and bioequivalence of two peroral beclobrate preparations], *Arzneimittelforschung*, **1987**, *37*, 1065–1068.

SAMPLE

Matrix: blood, urine
Sample preparation: 200 μL Plasma or 100 μL urine + 500 (plasma) or 300 (urine) mg NaCl + 50 μL 1 μg/mL clofibric acid in MeOH + 1 (plasma) or 0.3 (urine) mL pH 4 buffer + 5 mL n-hexane:EtOH 90:10, shake horizontally for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, add 50 μL toluene and evaporate it to remove traces of water. Reconstitute the residue in 500 μL dichloromethane, add 50 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane: pyridine 99:1, add 50 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 50 μL 1 mg/mL FLOPA, vortex, let stand at room temperature for 2 h, evaporate to dryness, reconstitute in 500 μL mobile phase, inject a 50 μL aliquot. (FLOPA is the corresponding amine hydrochloride from (+)-(S)-flunoxaprofen. Synthesis is as follows (protect from light). 500 mg (+)-(S)-Flunoxaprofen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, add 600 mg sodium azide dissolved in ice water with stirring, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate,

evaporate to dryness, dissolve in 1 mL toluene, evaporate to give crystals (mp 91°). Dissolve in ether, add 0.5 M HCl in ether, filter, dissolve solid in a small volume of MeOH, precipitate with ether, dry FLOPA over phosphorus pentoxide under vacuum (Pharm.Res. 1990, 7, 1262).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Gradient. A was n-hexane:chloroform:EtOH 100:10:0.75. B was n-hexane: chloroform:EtOH 100:10:20. A:B 100:0 for 10 min, 50:50 for 5 min, 100:0 for 5 min (stepwise).
Flow rate: 2
Injection volume: 50
Detector: F ex 305 em 355

CHROMATOGRAM

Retention time: 6 (-), 6.5 (+)
Internal standard: clofibric acid (8)
Limit of detection: 25 ng/mL

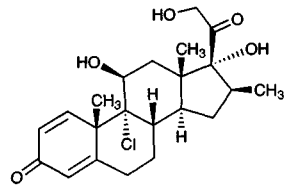
KEY WORDS

plasma; pharmacokinetics; chiral; derivatization; normal phase

REFERENCE

Mayer,S.; Mutschler,E.; Spahn-Langguth,H. Pharmacokinetic studies with the lipid-regulating agent beclobrate: enantiospecific assay for beclobric acid using a new fluorescent chiral coupling component (S-FLOPA), *Chirality*, **1991**, *3*, 35–42.

Beclomethasone



Molecular formula: $C_{22}H_{29}ClO_5$
Molecular weight: 408.92
CAS Registry No.: 4419-39-0, 5534-09-8 (beclomethasone dipropionate)
Merck Index: 1047

SAMPLE

Matrix: blood
Sample preparation: Add 30 mL EtOH to 50 mL plasma, extract with dichloromethane for 15 min, centrifuge at 2500 rpm for 5 min. Evaporate dichloromethane layer to dryness under vacuum at 30°, reconstitute residue in mobile phase and inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Alltima C18 (Alltech Associates, Australia) (A) or 250 × 4.6 5 μm Econosphere C18 (Alltech Associates, Australia) (B) or 150 × 4.6 5 μm Ultrasphere C8 (C)
Mobile phase: MeOH:MeCN:acetic acid:water 30.9:14.6:4.4:0.1 (A) or 25:20.5:4.4:0.1 (B) or 27.5:18:4.4:0.1 (C)
Flow rate: 1.3 (A, C) or 1.2 (B)
Injection volume: 100
Detector: UV 242

CHROMATOGRAM

Retention time: 4.9 (A), 5.5 (B), 3.3 (C) (beclomethasone); 23.0 (A), 25.9 (B), 17.6 (C) (beclomethasone dipropionate)

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS

plasma

REFERENCE

Foe,K.; Cheung,H.T.A.; Tattam,B.N.; Brown,K.F.; Seale,J.P. Degradation products of beclomethasone dipropionate in human plasma, *Drug Metab.Dispos.*, **1998**, *26*, 132–137.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 300 μL water + 6 mL diethyl ether, shake for 5 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen. Dissolve in 100 μL MeOH. Add 20 μL copper acetate solution and let stand at room temperature for 1 h. Add 80 μL diaminophthalhydrazide solution, heat at 80° for 110 min, cool, inject a 20 μL aliquot. (Prepare copper acetate solution by dissolving 700 mg copper(II) acetate in 10 mL water, dilute to 100 mL with MeOH, discard after 1 month. Diaminophthalhydrazide solution was 7.5 mM 4,5-diaminophthalhydrazide in 3.5 M hydrochloric acid containing 625 mM β-mercaptoethanol, discard after 5 h. Prepare 4,5-diaminophthalhydrazide dihydrochloride as follows. Reflux 316 g 4-nitrophthalic acid and 50 mL concentrated sulfuric acid in 500 mL MeOH for 10 h, recrystallize the product (dimethyl 4-nitrophthalate) from MeOH (mp 64-65E). Hydrogenate 47.8 g dimethyl 4-nitrophthalate in 300 mL MeOH over 13 g 5% platinum on carbon at an initial hydrogen pressure of 50 psi. When the calculated amount of hydrogen has been absorbed remove the catalyst and evaporate to dryness under reduced pressure, recrystallize the residue from aqueous MeOH to give dimethyl 4-aminophthalate (mp 83-84E). Stir 146.3 g dimethyl 4-aminophthalate in 1.4 L acetic anhydride at 60-70E for 2 h then leave over-

night, precipitate product with MeOH. Dry the product and rinse it with sodium carbonate solution, re-dry, recrystallize from benzene/MeOH (Caution! Benzene is a carcinogen!) to give dimethyl 4-acetamidophthalate (mp 138-140E). Add 100.4 g to 600 mL fuming (90%) nitric acid at 0-5E over 30 min, stir at 5-10E for 2.5 h, mix the reaction mixture with 800 mL cold dichloromethane, shake with crushed ice. Remove the organic layer and extract the aqueous layer with 200 mL cold dichloromethane. Combine the organic layers and wash them with ice water, cold sodium bicarbonate solution, and cold water. Dry over anhydrous magnesium sulfate, evapor ate to dryness under reduced pressure and, recrystallize repeatedly from MeOH to give dimethyl 4-acetamido-5-nitrophthalate (mp 123-124.5E). Hydrolyze dimethyl 4-acetamido-5-nitrophthalate to dimethyl 4-amino-5-nitrophthalate. Hydrogenate 20.3 g dimethyl 4-amino-5-nitrophthalate in 250 mL MeOH over 1 g 5% platinum on carbon at an initial hydrogen pressure of 50 psi, remove the catalyst, evaporate to dryness under reduced pressure at 25E, recrystallize from chloroform/dichloromethane to give dimethyl 4,5-diaminophthalate (mp 111.5-113E). Add 1.1 g dimethyl 4,5-diaminophthalate to 3 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) and 3 mL triethylamine in 20 mL MeOH, concentrate the resulting solution, triturate with benzene/MeOH, recrystallize from N,N'-dimethylacetamide/acetic acid to give 4,5-diaminophthalhydrazide (6,7-diamino-2,3-dihydrophthalazine-1,4-dione) (mp 407E) (J. Heterocycl. Chem. 1973, 10, 891), mix 4,5-diaminophthalhydrazide with a small amount of concentrated HCl, recrystallize from EtOH to give 4,5-diaminophthalhydrazide dihydrochloride.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSKgel ODS-120T (Tosoh, Japan)
Mobile phase: MeCN:tetrahydrofuran:100 mM pH 7.0 phosphate buffer 24:3:73
Flow rate: 1.0
Injection volume: 20
Detector: Chemiluminiscence following post-column reaction. The column effluent mixed with 20 mM hydrogen peroxide in water pumped at 1.0 mL/min and then with 30 mM potassium hexacyanoferrate(III) in 3.0 M NaOH pumped at 2.0 mL/min and flowed to the detector.

CHROMATOGRAM

Retention time: 52
Internal standard: beclomethasone

OTHER SUBSTANCES

Extracted: dexamethasone
Simultaneous: aldosterone, corticosterone, cortisone, 11-deoxycortisol, hydrocortisone, 18-hydroxycorticosterone, 18-hydroxydeoxycorticosterone, prednisolone, prednisone
Noninterfering: androstendione, cholesterol, estrone, estradiol, estriol, pregnenolone, progesterone
Interfering: betamethasone.

KEY WORDS

plasma; derivatization; beclomethasone is IS

REFERENCE

Ishida,J.; Sonezaki,S.; Yamaguchi,M.; Yoshitake,T., Determination of dexamethasone in plasma by high-performance liquid chromatography with chemiluminiscence detection, *Anal.Sci.*, **1993**, *9*, 319–322.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 750 μL 40 μg/mL IS in EtOH, add 8 mL dichloromethane, extract on a roller mixer for 30 min. Centrifuge at 2500 rpm for 10 min at 25°, collect the organic layer, evaporate to dryness at 30° under a stream of nitrogen. Reconstitute the residue in 1 mL mobile phase, centrifuge at 15000 rpm for 2 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Alltima C18 (Alltech)
Mobile phase: MeCN:MeOH:water:glacial acetic acid 8.8:65:26.2:0.175
Flow rate: 1.3
Injection volume: 100
Detector: UV 242

CHROMATOGRAM
Retention time: 15.4 (dipropionate)
Internal standard: dexamethasone-21-acetate (5.2)

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
plasma; serum

REFERENCE
Foe,K.; Brown,K.F.; Seale,J.P. Decomposition of beclomethasone propionate esters in human plasma, *Biopharm.Drug Dispos.*, **1998**, *19*, 1–8.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL 20 μg/mL cloprednol + 3 mL ether, shake 10 min, centrifuge at 3000 g, remove the organic phase and evaporate it to dryness under nitrogen. Take up the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: Nucleosil R 10 C 18
Mobile phase: MeOH:MeCN:water:acetic acid 400:100:200:1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Internal standard: cloprednol
Limit of detection: 500 ng/mL

KEY WORDS
for beclomethasone dipropionate; plasma

REFERENCE
Würthwein,G.; Rohdewald,P. Activation of beclomethasone dipropionate by hydrolysis to beclomethasone-17-monopropionate, *Biopharm.Drug Dispos.*, **1990**, *11*, 381–394.

SAMPLE
Matrix: blood, tissue
Sample preparation: Acidify plasma or lung tissue homogenate to pH 2 with 500 mM HCl, add 100 μL 20 μg/mL IS, extract with 8 mL dichloromethane. Evaporate the organic layer to dryness under vacuum, reconstitute in 120 μL MeOH:5% acetic acid 50:50, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS C18
Mobile phase: MeCN:MeOH:water 44:11:45
Flow rate: 1
Injection volume: 80
Detector: UV 242 or radioactivity

CHROMATOGRAM
Internal standard: hydrocortisone 21-S-propionate (JO 498)

OTHER SUBSTANCES
Extracted: metabolites, budesonide

KEY WORDS
for beclomethasone dipropionate; plasma; rat; lung; radiolabeled; pharmacokinetics

REFERENCE
Chanoine,F.; Grenot,C.; Heidmann,P.; Junien,J.L. Pharmacokinetics of butixocort 21-propionate, budesonide, and beclomethasone dipropionate in the rat after intratracheal, intravenous, and oral treatments, *Drug Metab.Dispos.*, **1991**, *19*, 546–553.

SAMPLE
Matrix: ileostomy effluent
Sample preparation: Dilute ileostomy effluent 1:2 by weight with water and mix with 100 µL 11 µg/mL 17-hydroxyprogesterone. Extract 3 g aliquot three times with 10 mL dichloromethane by shaking for 1 min and centrifuging at 2000 rpm for 2 min. Wash combined extracts successively with 2 mL 0.1 M NaOH and 4 mL water by shaking for 30 s and centrifuging for 1 min then dry the organic layer under air at 40°. Take up the extract in 1 mL MeOH, add 1.1 mL water and apply to C18 Bond Elut SPE cartridge. Wash with 10 mL water, wash with 5 mL MeOH:water 45:55, elute with 2 mL MeOH. Add 50 µL 20 µg/mL progesterone to the eluate, dry at 40°, take up in 100 µL MeOH, inject 10 µL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 µBondapak C18
Mobile phase: MeOH:50 mM pH 3.0 sodium phosphate buffer 55:45
Flow rate: 3
Injection volume: 10
Detector: UV 254 and 238

CHROMATOGRAM
Retention time: 21.3 (beclomethasone dipropionate)
Internal standard: 17-Hydroxyprogesterone (6.0) and progesterone (11.6)

OTHER SUBSTANCES
Extracted: beclomethasone alcohol, beclomethasone 17-monopropionate

KEY WORDS
SPE

REFERENCE
Levine,D.S.; Raisys,V.A.; Ainardi,V. Coating of oral beclomethasone dipropionate capsules with cellulose acetate phthalate enhances delivery of topically active antiinflammatory drug to the terminal ileum, *Gastroenterology*, **1987**, *92*, 1037–1044.

SAMPLE
Matrix: tissue
Sample preparation: 100 mg Tissue + 2 mL Ringer's pH 6.8 phosphate buffer + 2 mL EtOH, centrifuge, wash residue twice. Pool supernatant and washings and evaporate to dryness, take up in 400 µL EtOH, inject an aliquot.

HPLC VARIABLES
Guard column: Used but not specified

Column: μBondapak C18
Mobile phase: Gradient. MeOH:water 40:60 to 80:20, time not specified
Flow rate: 1.5
Detector: UV 254

OTHER SUBSTANCES

Extracted: beclomethasone monopropionate, beclomethasone, cyclomethasone

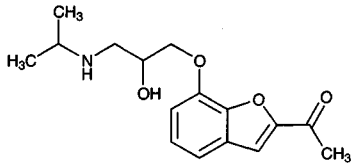
KEY WORDS

for beclomethasone dipropionate; lung

REFERENCE

Ronca-Testoni,S. Hydrolysis of cyclomethasone by the human lung, *Int.J.Clin.Pharmacol.Res.*, **1983**, *3*, 17–20.

Befunolol



Molecular formula: $C_{16}H_{21}NO_4$
Molecular weight: 291.35
CAS Registry No.: 39552-01-7
Merck Index: 1050

SAMPLE

Matrix: perfusate
Sample preparation: 50 µL Perfusate + 50 µL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 µL 50 µg/mL salicyl methionine in MeOH, centrifuge at 12000 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 45:55
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 500

CHROMATOGRAM

Internal standard: salicyl methionine

KEY WORDS

rabbit

REFERENCE

Sasaki,H.; Igarashi,Y.; Nagano,T.; Nishida,K.; Nakamura,J. Different effects of absorption promoters on corneal and conjunctival penetration of ophthalmic β-blockers, *Pharm.Res.*, **1995**, *12*, 1146–1150.

SAMPLE

Matrix: solutions
Sample preparation: 50 µL Solution + 50 µL pH 7.4 PBS + 100 µL 50 µg/mL salicylmethionine in MeOH, centrifuge at 12000 g for 10 min, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 45:55
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 500

CHROMATOGRAM

Internal standard: salicylmethionine

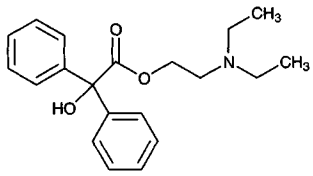
KEY WORDS

buffer; earle's balanced salt solution

REFERENCE

Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

Benactyzine



Molecular formula: $C_{20}H_{25}NO_3$
Molecular weight: 327.42
CAS Registry No.: 302-40-9, 57-37-4 (HCl)
Merck Index: 1055
Lednicer No.: 1 93

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 1.99

OTHER SUBSTANCES

Simultaneous: buclizine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

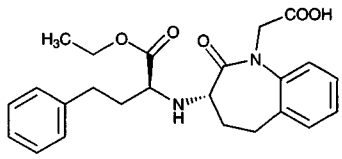
KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

Benazepril



Molecular formula: $C_{24}H_{28}N_2O_5$
Molecular weight: 424.50
CAS Registry No.: 86541-75-5, 86541-74-4 (HCl)
Merck Index: 1058
Lednicer No.: 5 135

SAMPLE

Matrix: formulations
Sample preparation: Add MeOH:water 50:50 to powdered capsules or tablets so as to give a benazepril concentration of ca. 20 μg/mL, stir for 15 min, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Hypersil ODS
Mobile phase: MeCN:THF:20 mM pH 2.5 sodium heptanesulfonate 45.6:2.4:52
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM

Retention time: 15.5

OTHER SUBSTANCES

Simultaneous: ramipril

KEY WORDS

capsules; tablets

REFERENCE

Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 17.003

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder. Weigh out an amount equivalent to 25 mg benazepril, extract with MeOH, filter. Mix 100-500 μL filtrate with 200 μL 4 mg/mL IS in MeOH, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 211

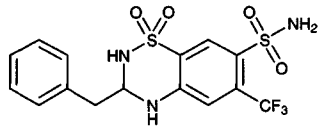
CHROMATOGRAM
Retention time: 18.53
Internal standard: enalapril (8.58)
Limit of detection: 5 μg/mL
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: cilazapril

KEY WORDS
tablets

REFERENCE
Gumieniczek,A.; Przyborowski,L. Determination of benazepril and cilazapril in pharmaceuticals by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2135–2142.

Bendroflumethiazide



Molecular formula: $C_{15}H_{14}F_3N_3O_4S_2$
Molecular weight: 421.42
CAS Registry No.: 73-48-3
Merck Index: 1064
Lednicer No.: 2 358

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 2 mL 10 mM NaOH + 2 mL 10 mM HCl, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 50 µL 10 mM NaOH, vortex for 25 s, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: Isopropanol:water:acetic acid 17:82:1
Flow rate: 2
Injection volume: 20
Detector: UV 269

CHROMATOGRAM

Retention time: 14.4
Internal standard: bendroflumethiazide

OTHER SUBSTANCES

Extracted: trichlormethiazide

KEY WORDS

plasma; silanize glassware; bendroflumethiazide is IS

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 18.632

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in solvent, inject an aliquot. (Solvent was 750 mg KCl in 10 mL 1 M HCl, add 400 mL water, add 400 mL MeOH, make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 4 7 μm Nucleosil-100 phenyl
Column: 300 × 4 7 μm Nucleosil-100 phenyl
Mobile phase: MeOH:water 40:60
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: hydroflumethiazide, degradation products

REFERENCE
Frontini,R.; Mielck,J.B. Determination and quantitation of bendroflumethiazide and its degradation products using HPLC, *J.Liq.Chromatogr.*, **1992**, *15*, 2519–2528.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3001 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 272

CHROMATOGRAM
Retention time: 19, 21.5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 80:20, inject a 6 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 10 μm LiChrosorb RP-8
Column: 100 × 4.6 5 μm Spheri RP-18 (Brownlee)
Mobile phase: MeOH:water 80:20 containing 2 g/L lithium perchlorate
Flow rate: 0.5
Injection volume: 6
Detector: E, ESA Model 5100A Coulochem, model 5020 guard cell +950 mV, Model 5010 analytical cell + 400 mV, palladium reference electrode, following post-column photolysis. The effluent from the column flowed through a 20 m × 0.3 mm coil of PTFE tubing and was irradiated at 254 nm with a Sylvania GTE 8 W low-pressure lamp.

CHROMATOGRAM
Retention time: 5
Limit of detection: 267 ng/mL

OTHER SUBSTANCES
Also analyzed: butizide, chlorthalidone, ethacrynic acid, furosemide, hydrochlorothiazide

KEY WORDS
post-column reaction

REFERENCE
Macher,M.; Wintersteiger,R. Improved electrochemical detection of diuretics in high-performance liquid chromatographic analysis by postcolumn on-line photolysis, *J.Chromatogr.A*, **1995**, *709*, 257–264.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica
Mobile phase: Heptane:isopropanol:diethylamine 80:20:0.1
Flow rate: 1
Injection volume: 1000
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.34

KEY WORDS
chiral; α 1.17

REFERENCE
Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, *18*, 1521–1532.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 500 mg solid sodium bicarbonate, mix, add 2 mL 10 mM NaOH, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 100 μL MeOH, vortex for 25 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 5:35:59:1
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 10.7
Internal standard: bendroflumethiazide

OTHER SUBSTANCES

Extracted: trichlormethiazide

KEY WORDS

silanize glassware; bendroflumethiazide is IS

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM

Retention time: 6.8
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, chlorthalidone, clopamide, methyclothiazide, furosemide, metolazone, mefruside, cyclopenthiazide, bumetanide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, in-

domethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE

Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 15 (A), 15.4 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

Interfering: polythiazide

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 1 mL 10 mM HCl + 2000 ng bendroflumethiazide, extract with 5 mL ethyl acetate, centrifuge at 3000 rpm for 5 min. Remove the organic layer and dry it under a stream of nitrogen at 40°. Reconstitute with 100 μL MeOH, inject a 2 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 μm Hypersil ODS

Mobile phase: Gradient. MeOH: 50 mM ammonium acetate from 10:90 to 60:40 over 10 min, maintain at 60:40 for 10 min.
Column temperature: 40
Flow rate: 0.3
Injection volume: 2
Detector: F ex 223 em 415 or UV 230

CHROMATOGRAM
Retention time: 8.6
Internal standard: bendroflumethiazide

OTHER SUBSTANCES
Extracted: bumetanide (F ex 231 em 426 or UV), furosemide (UV), piretanide (UV), cyclopenthiazide (UV), etozolin (UV), canrenone (UV)

KEY WORDS
bendroflumethiazide is IS

REFERENCE
Gradeen,C.Y.; Billay,D.M.; Chan,S.C. Analysis of bumetanide in human urine by high-performance liquid chromatography with fluorescence detection and gas chromatography/mass spectrometry, *J.Anal.Toxicol.*, **1990**, *14*, 123–126.

SAMPLE
Matrix: urine
Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 µL MeCN/water, inject a 10-20 µL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 µm SGE 100 GL-4 C18P (Scientific Glass Engineering)
Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 4.5:10.5:85:0.5
Flow rate: 0.8 or 1
Injection volume: 10-20
Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Extracted: amiloride, chlorthalidone, triamterene, furosemide, benzthiazide

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Direct injection.

HPLC VARIABLES
Guard column: 35 × 4 5 µm Spherisorb ODS-2
Column: 120 × 4 5 µm Spherisorb ODS-2
Mobile phase: MeOH:50 mM sodium dodecyl sulfate 5:95

Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 224

CHROMATOGRAM
Retention time: 15.2
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: chlorthalidone

REFERENCE
Bonet Domingo,E.; Medina Hernández,M.J.; Ramis Ramos,G.; Garcia Alvarez-Coque,M.C. High-performance liquid chromatographic determination of diuretics in urine by micellar liquid chromatography, *J.Chromatogr.*, **1992**, *582*, 189–194.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 270

CHROMATOGRAM
Retention time: 18.8
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Recon-

stitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 7.0
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, probenecid, spironolactone, torsemide, triamterene
Interfering: polythiazide, ethacrynic acid

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine
Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 9.8
Limit of detection: 20 ng/mL.

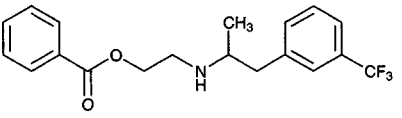
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Benfluorex



Molecular formula: $C_{19}H_{20}F_3NO_2$
Molecular weight: 351.37
CAS Registry No.: 23602-78-0, 23642-66-2 (HCl)
Merck Index: 1066

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.378

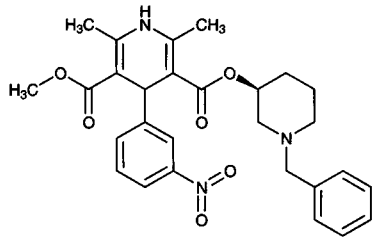
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Benidipine



Molecular formula: $C_{28}H_{31}N_3O_6$
Molecular weight: 505.57
CAS Registry No.: 105979-17-7
Merck Index: 1071

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Urine, bile. Inject urine and bile directly. Plasma. Add an equal volume of MeCN, centrifuge, remove supernatant and evaporate it to dryness. Reconstitute residue in MeCN:10 mM pH 5 ammonium acetate 20:80.

HPLC VARIABLES

Column: 300 × 8 Unisil Q5C18 (Gaschro Kogyo)
Mobile phase: Gradient. A was MeCN:10 mM pH 5 ammonium acetate 20:80. B was MeCN:10 mM pH 5 ammonium acetate 80:20. A:B from 100:0 to 92:8 over 4 min, to 83:17 over 4 min, to 75:25 over 4 min, to 63:37 over 4 min, to 55:45 over 8 min, to 50:50 over 8 min, to 42:58 over 4 min, to 0:100 over 9 min, hold at 0:100 for 25 min.
Flow rate: 2 for 36 min, then 3
Detector: Radioactivity

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; semi-preparative; rat; dog; radiolabelled

REFERENCE

Kobayashi,H.; Okumura,S.; Kosaka,Y.; Kobayashi,S.; Inoue,A.; Oka,T.; Nakamizo,N. Identification of benidipine hydrochloride metabolites in rats and dogs, *Arzneimittelforschung*, **1988**, *38*, 1753–1756.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 50 mg in 50 mL MeOH:water 70:30, remove a 10 mL aliquot and add it to 10 mL 1 mg/mL diphenylamine in MeOH, make this mixture up to 100 mL with MeOH:water 70:30, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18
Mobile phase: MeOH:100 mM pH 5 ammonium acetate 70:30 (MeOH:50 mM pH 3.5 phosphate buffer:diisopropylamine 60:40:1 at 0.7 mL/min to detect other diastereomer)
Flow rate: 1
Injection volume: 10
Detector: UV 236

CHROMATOGRAM

Retention time: 10
Internal standard: diphenylamine (6)

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Suzuki,H.; Ono,E.; Ueno,H.; Takemoto,Y.; Nakamizo,N. Physico-chemical properties and stabilities of the highly potent calcium antagonist benidipine hydrochloride, *Arzneimittelforschung*, **1988**, *38*, 1671–1676.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out solid dispersion or solid dispersion granules equivalent to 20 mg benidipine hydrochloride, add 200 mL water, 100 mM hydrochloric solution, or McIlvaine buffer (pH 4.0, 6.0), filter (0.2 μm), dilute with MeOH, inject an aliquot.

HPLC VARIABLES

Column: C18 (YMC-Pack ODS-A)
Mobile phase: MeOH:THF:50 mM pH 3.0 phosphate buffer 27:8:65
Flow rate: 1
Detector: UV 237

CHROMATOGRAM

Internal standard: benzoin

KEY WORDS

solid dispersions; solid dispersion granules

REFERENCE

Suzuki,H.; Miyamoto,N.; Masada,T.; Hayakawa,E.; Ito,K. Solid dispersions of benidipine hydrochloride. I. Preparations using different solvent systems and dissolution properties, *Chem.Pharm.Bull.*, **1996**, *44*, 364–371.

SAMPLE

Matrix: solutions

Sample preparation: Direct injection of a MeOH solution containing 200-1000 ng.

HPLC VARIABLES

Column: 250 × 4.6 Sumchiral OA-4500 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:10:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 33 (+), 42(-) (α = 1.30)

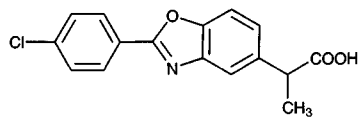
KEY WORDS

chiral

REFERENCE

Ohkubo,T.; Uno,T.; Sugawara,K. Enantiomer separation of dihydropyridine derivative calcium antagonists by high-performance liquid chromatography with chiral stationary phases, *J.Chromatogr.A*, **1994**, *659*, 467–471.

Benoxaprofen



Molecular formula: $C_{16}H_{12}ClNO_3$
Molecular weight: 301.73
CAS Registry No.: 67434-14-4
Merck Index: 1075

SAMPLE

Matrix: bile, blood
Sample preparation: 10 μL Plasma, or bile + 180 μL MeCN + 30 μL 100 μL/mL IS in DMSO + 30 μL water, vigorously mix. Centrifuge at 15000 g for 10 min at 4°, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.6 SUMICHIRAL OA 2500 (Sumika Chemical Analysis Service, Japan)
Column: 250 × 4.6 SUMICHIRAL OA 2500 (Sumika Chemical Analysis Service, Japan)
Mobile phase: MeOH containing 40 mM ammonium acetate (plasma) or MeCN:MeOH: water 15:85:5 containing 10 mM ammonium acetate (bile)
Flow rate: 1.0
Injection volume: 10
Detector: F ex 315, em 365

CHROMATOGRAM

Retention time: 10.3 (R, plasma) 11.8 (S, plasma), 22.5 (R, bile) 26.0 (S, bile)
Internal standard: naproxen methyl ester (4.4) (plasma), (4.3) (bile)
Limit of detection: 10 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat; chiral; pharmacokinetics

REFERENCE

Mohri,K.; Okada,K.; Benet,L.Z. Stereoselective metabolism of benoxaprofen in rats. Biliary excretion of benoxaprofen taurine conjugate and glucuronide, *Drug Metab.Dispos.*, **1998**, *26*, 332–337.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: 10 μL Plasma, bile, or urine + 180 μL MeCN + 30 μL 100 μL/mL IS in DMSO + 30 μL water, mix vigorously. Centrifuge at 15000 g for 10 min at 4°, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Capcell Pak C18 (Shiseido, Japan)
Column: 250 × 4.6 5 μm Capcell Pak C18 (Shiseido, Japan)
Mobile phase: MeCN:THF:10 mM tetrabutylammonium hydrogen sulfate buffer 35:35:100
Flow rate: 1.3
Injection volume: 10
Detector: F ex 315 em 365

CHROMATOGRAM

Retention time: 17.5
Internal standard: naproxen methyl ester (13.2)

Limit of detection: 10 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

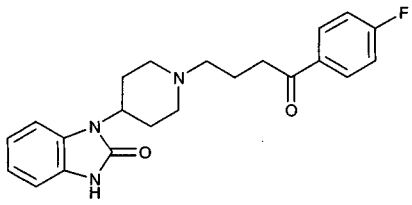
KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Mohri,K.; Okada,K.; Benet,L.Z. Stereoselective metabolism of benoxaprofen in rats. Biliary excretion of benoxaprofen taurine conjugate and glucuronide, *Drug Metab.Dispos.*, **1998**, *26*, 332–337.

Benperidol



Molecular formula: $C_{22}H_{24}FN_3O_2$
Molecular weight: 381.45
CAS Registry No.: 2062-84-2
Merck Index: 1077
Lednicer No.: 2 290

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 246

CHROMATOGRAM

Retention time: 4.62

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

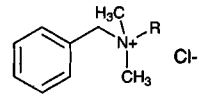
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, peri-

cyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Benzalkonium chloride



R = alkyl

CAS Registry No.: 8001-54-5
Merck Index: 1086

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeCN:buffer 50:50 (Buffer was 13.6 g/L sodium acetate, pH adjusted to 4.5 with glacial acetic acid.)
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 8 (C12), 11 (C14), 14 (C16)

OTHER SUBSTANCES
Simultaneous: demecarium

KEY WORDS
ophthalmic solution

REFERENCE
Cohn,L.J.; Greely,V.J.; Tibbetts,D.L. Determination of demecarium bromide and related compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *321*, 401–405.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 6.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: domiphen bromide, thimerosal, xylometazoline

KEY WORDS
nasal drops

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: formulations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with THF:mobile phase 70:30 then with water. Add 10 mL formulation to the SPE cartridge, wash with water, elute with 3 mL THF:mobile phase 70:30 then with 3 mL mobile phase, mix the eluates, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax Stablebond CN
Mobile phase: THF:water:triethylamine 150:250:2, pH adjusted to 3.0 ± 0.1 with phosphoric acid
Column temperature: 40
Flow rate: 2
Injection volume: 100
Detector: UV 215

CHROMATOGRAM
Retention time: 3.5 (C12), 6.5 (C14), 10 (C16)

KEY WORDS
SPE; eye

REFERENCE
Elrod,L.,Jr.; Golich,T.G.; Morley,J.A. Determination of benzalkonium chloride in eye care products by high-performance liquid chromatography and solid-phase extraction or on-line column switching, *J.Chromatogr.*, **1992**, *625*, 362–367.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 1 mL Supelcoclean cyano SPE cartridge with 2 mL MeCN and 2 mL water. Add 4 mL formulation to the SPE cartridge, wash with 2 mL MeCN:buffer 30:70, elute with 5 mL mobile phase, make up eluate to 10 mL with water, inject a 100 μL aliquot. (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.).

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.)
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 4.5 (C10), 5.5 (C12), 6.5 (C14), 7.5 (C16), 8.5 (C18)

OTHER SUBSTANCES
Also analyzed: tyloxapol

KEY WORDS
ophthalmic solutions; eye; SPE

REFERENCE
Fan,T.Y.; Wall,G.M. Determination of benzalkonium chloride in opthalmic solutions containing tyloxapol by solid-phase extraction and reversed-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1993**, *82*, 1172–1174.

SAMPLE
Matrix: formulations
Sample preparation: 2 mL Sample + 1 mL 200 μg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 μm), inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Technosphere RP C-8 (HPLC Technology)
Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 3.15 (C12), 4.21 (C14), 5.78 (C16)
Internal standard: emetine (1.75)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: naphazoline, tetrahydrozoline

KEY WORDS
nasal; ophthalmic

REFERENCE
Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzauti,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, *49*, 751–754.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:buffer 65:35 (Buffer was 50 mM KH_2PO_4 and 57 mM sodium hexanesulfonate, adjusted to pH 6.3 with 1 M NaOH.)
Flow rate: 1.8
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 7.5 (C12), 12.1 (C14)

OTHER SUBSTANCES
Noninterfering: phenylephrine

KEY WORDS
ophthalmic solution; stability-indicating

REFERENCE
Parhizkari,G.; Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of benzalkonium chloride in phenylephrine HCl 10% ophthalmic solution, *J.Liq.Chromatogr.*, **1995**, *18*, 553–563.

SAMPLE
Matrix: formulations

Sample preparation: Dilute 0.5% ophthalmic solution 1:5 with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm CPS Hypersil-1 cyano
Mobile phase: MeCN:buffer 65:35 (Buffer was 50 mM sodium propionate adjusted to pH 5.3 with concentrated sulfuric acid.)
Flow rate: 1.3
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 10.0 (C12), 11.7 (C14)

KEY WORDS
ophthalmic solutions; stability-indicating

REFERENCE
Parhizkari,G.; Delker,G.; Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of benzalkonium chloride in 0.5% Tramadol ophthalmic solution, *Chromatographia*, **1995**, *40*, 155–158.

SAMPLE
Matrix: sewage
Sample preparation: Condition a C18ec SPE cartridge (Macherey-Nagel) with three bed volumes of MeOH and three bed volumes of water. Allow sewage to settle, add an 8 mL aliquot of the supernatant to the SPE cartridge, wash with 3 bed volumes of water, wash with 2 bed volumes of ethyl acetate, elute with 2 bed volumes of 1% calcium chloride in MeOH:ethyl acetate 50:50, inject a 15 μL aliquot of the eluate. (For all steps involving the SPE cartridge the flow rate should be 3 mL/min.)

HPLC VARIABLES
Column: 250 × 4 5 μm Partisil PAC CN-NH2
Mobile phase: Chloroform:MeOH 80:20
Column temperature: 15
Flow rate: 1
Injection volume: 15
Detector: F ex 383 em 459 following post-column extraction. The column effluent mixed with 37 μg/mL sodium 9,10-dimethoxyanthracene-2-sulfonate in water pumped at 0.3 mL/min and the mixture flowed to a phase separator. The organic phase flowed to the detector and the aqueous phase was discarded. (9,10-Dimethoxyanthracene-2-sulfonate forms a hydrophobic fluorescent ion-pair with benzalkonium chloride.)

CHROMATOGRAM
Retention time: 2.49
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: dimethyldidecylammonium chloride (DDMAC)

KEY WORDS
post-column reaction; post-column extraction; SPE

REFERENCE
Kümmerer,K.; Eitel,A.; Braun,U.; Hubner,P.; Daschner,F.; Mascart,G.; Milandri,M.; Reinthaler,F.; Verhoef,J. Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and high-performance liquid chromatography with post-column ion-pairing and fluorescence detection, *J.Chromatogr.A*, **1997**, *774*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM pH 5 sodium acetate buffer 60:40
Flow rate: 1
Detector: UV 254

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Prince,S.J.; Allen,L.V. Analysis of benzalkonium chloride and its homologs: HPLC vs HPCE (Abstract APQ 1093), *Pharm.Res.*, **1996**, *13*, S26–S26.

SAMPLE
Matrix: solutions

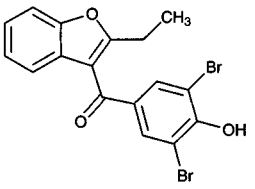
HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM pH 5 sodium acetate buffer 60:40
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Limit of quantitation: 25 μg/mL

KEY WORDS
run time 25 min; for C12; C14; C16 homologs; comparison with capillary electrophoresis

REFERENCE
Prince,S.J.; McLaury,H.J.; Allen,L.V.,Jr. Comparison of HPCE and HPLC for the separation and quantitation of benzalkonium chloride homologs (Abstract 3012), *Pharm.Res.*, **1997**, *14*, S463–S464.

Benzbromarone



Molecular formula: $C_{17}H_{12}Br_2O_3$
Molecular weight: 424.09
CAS Registry No.: 3562-84-3
Merck Index: 1093
Lednicer No.: 2 354

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM

Retention time: 26.075

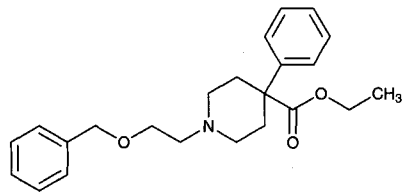
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Benzethidine



Molecular formula: $C_{23}H_{29}NO_3$
Molecular weight: 367.49
CAS Registry No.: 3691-78-9

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ran-

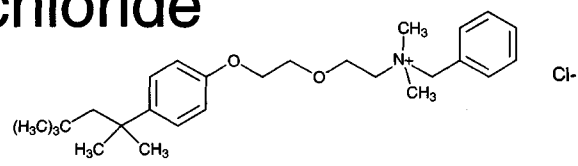
itidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Benzethonium chloride

Molecular formula: $C_{27}H_{42}ClNO_2$
Molecular weight: 448.09
CAS Registry No.: 121-54-0
Merck Index: 1103



SAMPLE
Matrix: saliva
Sample preparation: Collect sample on Periopaper strip (filter paper), add paper to 100 µL MeCN:water:glacial acetic acid 55:44.8:0.2 containing 7 mM sodium lauryl sulfate, vortex for 1 min, sonicate for 20 min, vortex, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 µm C18 ODS-B Exsil (HiChrome)
Mobile phase: MeCN:0.2% acetic acid 55:45 containing 5 mM sodium lauryl sulfate
Flow rate: 0.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: benzethonium

OTHER SUBSTANCES
Extracted: chlorhexidine

KEY WORDS
narrow-bore; benzethonium is IS

REFERENCE
Medlicott,N.J.; Ferry,D.G.; Tucker,I.G.; Rathbone,M.J.; Holborow,D.W.; Jones,D.S. High performance liquid chromatographic (HPLC) assay for the determination of chlorhexidine in saliva film, *J.Liq.Chromatogr.*, **1994**, *17*, 1605–1620.

SAMPLE
Matrix: tissue
Sample preparation: Mix 10 g minced fish or squid + 50 mL 96% EtOH + 50 µL 1-hexanesulfonic acid + 500 µL 2 M HCl, mix thoroughly for 1 h, filter, extract residue with 50 mL 96% EtOH,filter, press filter cake, wash with 25 mL EtOH. Combine filtrates, evaporate under vacuum below 70°, take up residue in 500 µL 2 M HCl + 500 µL MeOH + 50 µL 1-hexanesulfonic acid + 5 mL light petroleum (bp 30-40°), agitate for 2 min, pour out of flask, rinse flask with the same mixture, rinse flask with light petroleum. Combine all extracts, heat at 70° in a water bath until the organic layer disappears, add 2 mL dichloromethane:light petroleum 50:50, mix for 1 min, inject a 5-20 µL aliquot of the organic phase.

HPLC VARIABLES
Column: 300 × 3.9 µBondapak CN
Mobile phase: MeCN:100 mM ammonium acetate 75:25, containing 15 mL/L PIC B6 (Waters)
Flow rate: 1
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5
Limit of detection: 5-10 ppm

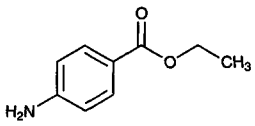
KEY WORDS

fish; squid

REFERENCE

Reuvers,T.B.A.; Ortíz,G.; Ramos,M.; Martín de Pozuelo,M. Rapid high-performance liquid chromatographic method for the determination of bencetonium chloride residues in fish products; confirmation by thin-layer chromatography, *J.Chromatogr.*, **1989**, *467*, 321–326.

Benzocaine



Molecular formula: $C_9H_{11}NO_2$
Molecular weight: 165.19
CAS Registry No.: 94-09-7
Merck Index: 1116
Lednicer No.: 1 9

SAMPLE

Matrix: bile, blood, tissue

Sample preparation: Plasma, bile. Add three 250 μL portions of MeOH to 250 μL plasma and three 150 μL portions of MeOH to150 μL bile, centrifuge. Remove MeOH from the supernatant using vacuum centrifugal evaporation. Dilute the residue to 1 mL with sample solvent. Filter (0.22 μm) and inject a 100 μL aliquot. Tissue. Homogenize 1.1.g white muscle, 640 mg liver, 250 mg trunk, or 170 mg head kidneys with 5 volumes of 100 mM acetic acid. Homogenize 500 mg red muscle and 320 mg skin with 5 volumes of 100 mM and 25 mM sodium acetate at pH 4.5, respectively. Add five volumes of MeOH to the homogenates, shake horizontally for 15 min. Centrifuge at 3000 g at 15° for 15 min and remove MeOH using vacuum centrifugal evaporation. Acidify the residue with 10% acetic acid to a final acetic acid concentration of 1% (skin to 2.5% acetic acid concentration) and add to a 3 mL Cyanopropyl (Supelco) SPE cartridge, elute with MeOH. Evaporate the MeOH under nitrogen at 40°. Reconstitute the residue with 500 μL sample solvent. Filter (0.22 μm) and inject a 100 μL aliquot. (Sample solvent was MeOH:water:acetic acid 15:84:1.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Lichrosorb Diol
Column: 150 × 4.6 3 μm LC-18-DB Supelco
Mobile phase: Gradient. A was MeOH:water:acetic acid 5:94:1. B was MeOH:water:acetic acid 55:44:1. A:B 100:0 for 3 min, to 0:100 over 5 min, maintain at 0:100 for 15 min, return to 100:0 over 1 min.
Injection volume: 100
Detector: UV 286

CHROMATOGRAM

Retention time: 15.5
Limit of quantitation: 1 nM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; catfish; head kidney; liver; plasma; red muscle; skin; trunk; white muscle; kidney; muscle

REFERENCE

Szoke,A.; Hayton,W.L.; Schultz,I.R. Quantification of benzocaine and its metabolites in channel catfish tissues and fluids by HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 69–75.

SAMPLE

Matrix: blood

Sample preparation: Centrifuge 300 μL whole blood at 5100 RCF for 8 min at 0°. Separate plasma from erythrocytes within 30 min after withdrawing blood. Warm sample to room temperature. Vortex 130 mg (125 μL) plasma with 250 μL MeOH for 1 min, centrifuge at 10 400 RCF for 15 min at 5°. Filter (0.45 μ PTFE) supernatant. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 23 × 4.0 5 μm spherical C18 with 17% carbon load, ODS type A (YMC, Inc., Wilmington)
Column: 150 × 4.6 5 μm spherical C18 with 17% carbon load, ODS type A (YMC, Inc., Wilmington)
Mobile phase: MeOH: 25 mM KH_2PO_4 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 286

CHROMATOGRAM

Retention time: 5.9
Limit of detection: 10 ng/mL
Limit of quantitation: 37 ng/mL

OTHER SUBSTANCES

Extracted: ethyl p-acetamidobenzoate

KEY WORDS

plasma; trout

REFERENCE

Bernardy,J.A.; Coleman,K.S.; Stehly,G.R.; Gingerich,W.H. Determination of benzocaine in rainbow trout plasma, *J.AOAC Int.*, **1996**, *79*, 623–627.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 291

CHROMATOGRAM

Retention time: 3.51
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebuto-

lol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100

Column: 125 × 4 3 μm Spherisorb ODS-1

Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.

Flow rate: 0.7

Injection volume: 15

Detector: UV 210

CHROMATOGRAM

Retention time: 15.9

OTHER SUBSTANCES

Simultaneous: acetaminophen, acetylcodeine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE

Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out 50 mg formulation, add 5 mL 1.5 mg/mL benzophenone in MeOH, make up to 50 mL with MeOH. Dilute 1 mL of this solution to 10 mL with MeOH, filter (0.45 μm PTFE membrane), inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 30 mm long Brownlee guard column
Column: 220 × 4.6 5 μm C18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 1.8
Internal standard: benzophenone (3.3)
Limit of quantitation: 10000 ng/mL

OTHER SUBSTANCES

Simultaneous: benzyl benzoate

KEY WORDS

dermatological preparations

REFERENCE

Gigante,B.; Barros,A.M.V.; Teixeira,A.; Marcelo-Curto,M.J. Separation and simultaneous high-performance liquid chromatographic determination of benzocaine and benzyl benzoate in a pharmaceutical preparation, *J.Chromatogr.*, **1991**, *549*, 217–220.

SAMPLE

Matrix: formulations
Sample preparation: Shake 2 g powder with 10 mL EtOH for 1 h, directly inject 1 mL of this solution using a syringe-coupled nylon filter (Teknokroma).

HPLC VARIABLES

Column: 125 × 4 5 μm Aluspher RP Select B (E. Merck) (alumina particles bonded with polybutadiene)
Mobile phase: MeOH:water:diammonium phosphate 30:70:0.2 (v:v:w), pH 8.2
Flow rate: 1
Injection volume: 20
Detector: UV 191

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: menthol, tyrothricin
Noninterfering: gramicidin

REFERENCE

Caraballo,I.; Fernandez-Arevalo,M.; Holgado,M.-A.; Vela,M.-T.; Rabasco,A.-M. A rapid HPLC method for the quantification of tyrothricin, menthol, and benzocaine in pharmaceutical formulations, *J.Pharm.Sci.*, **1994**, *83*, 1147–1149.

SAMPLE

Matrix: formulations

Sample preparation: Grind (Sorvall Omni-Mixer) and mix 14 lozenges for 2 min. Add 6.3 g powder to 15 mL mobile phase, sonicate (Bandelin Sonorex K 52) for 30 min, filter (paper), make up filtrate to 25 mL, dilute 13-fold, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau)
Mobile phase: MeOH:10 mM pH 3.31 KH_2PO_4 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: tyrothricin
Noninterfering: menthol

KEY WORDS

lozenges

REFERENCE

Ortiz-Boyer,F.; Tena,M.T.; Luque de Castro,M.D.; Valcárel,M. Development and validation of chromatographic methods (HPLC and GC) for the determination of the active components (benzocaine, tyrothricin and menthol) of a pharmaceutical preparation, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1297–1303.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 5 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 5
Detector: UV 305

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: butamben, lidocaine, pramoxine, procaine, tetracaine

REFERENCE

Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 569–570.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: butacaine, lidocaine, bupivacaine, tetracaine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.262

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.25 (A), 6.23 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: sunscreen
Sample preparation: Weigh out 1 g sunscreen, add 2-10 mL mobile phase, stir magnetically for 5 min, filter (0.45 μm Millex-HV), inject an aliquot.

HPLC VARIABLES

Column: 200 × 5 5 μm Nucleosil C18
Mobile phase: MeCN:15 mM phosphoric acid 18:82

Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Limit of detection: 500 ng/mL

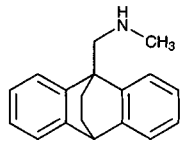
OTHER SUBSTANCES

Extracted: p-aminobenzoic acid

REFERENCE

Bruze,M.; Gruvberger,B.; Thulin,I. PABA, benzocaine, and other PABA esters in sunscreens and after-sun products, *Photodermatol.Photoimmunol.Photomed.*, **1990**, *7*, 106–108.

Benzoctamine



Molecular formula: $C_{18}H_{19}N$
Molecular weight: 249.36
CAS Registry No.: 17243-39-9, 10085-81-1 (HCl)
Merck Index: 1117
Lednicer No.: 2 220

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

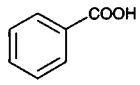
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Benzoic acid



Molecular formula: $C_7H_6O_2$
Molecular weight: 122.12
CAS Registry No.: 65-85-0
Merck Index: 1122

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: hydroquinine, quinine, saccharin

KEY WORDS
tonic water; soft drinks

REFERENCE
Valenti,L.P. Liquid chromatographic determination of quinine, hydroquinine, saccharin, and sodium benzoate in quinine beverages, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 782–784.

SAMPLE
Matrix: beverages
Sample preparation: Filter sample.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Hiasil C18 (Higgins)
Mobile phase: MeOH:25 mM phosphate buffer 45:55, pH 3.0
Flow rate: 1.0
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 6.0
Limit of detection: 0.57 mg/mL

OTHER SUBSTANCES
Simultaneous: aspartame, caffeine

KEY WORDS
comparison with UV spectrophotometry and capillary electrophoresis; soft drinks

REFERENCE
McDevitt,V.L.; Rodriguez,A.; Williams,K.R. Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, *J.Chem.Educ.*, **1998**, *75*, 625–629.

SAMPLE
Matrix: beverages, syrup
Sample preparation: Dilute syrup ten fold. Filter (0.45 μm) beverages and diluted syrup, inject a 10-20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 20:5:75
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: acesulfame, caffeine, dulcin, p-hydroxybenzoic acid, saccharin, vanillin

REFERENCE
Veerabhadrarao,M.; Narayan,M.S.; Kapur,O.; Sastry,C.S. Reverse phase liquid chromatographic determination of some food additives, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 578–582.

SAMPLE
Matrix: bile, blood
Sample preparation: Blood, plasma. 250 μL Blood or plasma + 50 μL 16 μg/mL methoxybenzoic acid in water + 800 μL MeCN, mix, centrifuge. Remove the supernatant and dry it under a stream of nitrogen, reconstitute the residue in 200 μL MeCN:0.5% acetic acid 10:90, centrifuge, inject an aliquot. Bile. Inject bile directly.

HPLC VARIABLES
Guard column: 22 × 3.4 37-50 μm C18 (Waters)
Column: 300 × 3.9 10 μm Ultrasphere
Mobile phase: Gradient. MeCN:0.5% acetic acid 10:90 for 10 min then to 72.5:27.5 over 2 min, maintain at 72.5:27.5 for 8 min, return to initial conditions over 1 min, re-equilibrate for 4 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 17.8
Internal standard: methoxybenzoic acid (19.1)

OTHER SUBSTANCES
Extracted: hippuric acid, benzoyl glucuronide

KEY WORDS
plasma

REFERENCE
Chiba,M.; Poon,K.; Hollands,J.; Pang,K.S. Glycine conjugation activity of benzoic acid and its acinar localization in the perfused rat liver, *J.Pharmacol.Exp.Ther.*, **1994**, *268*, 409–416.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 mL MeCN and 2 mL pH 3 aqueous acetic acid. Dilute 1 mL serum with 1 mL pH 3 aqueous acetic

acid, add to SPE cartridge, wash with 500 μL pH 3 aqueous acetic acid, elute with 1 mL MeCN acidified to pH 2.5 with acetic acid, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: Capcell Pak C-18 AG-120 (Shiseido)
Mobile phase: MeCN:water 20:80
Flow rate: 1.2
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 5.8

KEY WORDS
serum; dental material; methyl methacrylate polymer; plexiglass; horse; SPE

REFERENCE
Shintani,H.; Tsuchiya,T.; Hata,Y.; Nakamura,A. Solid phase extraction and HPLC analysis of toxic components eluted from methyl methacrylate dental materials, *J.Anal.Toxicol.*, **1993**, *17*, 73–78.

SAMPLE
Matrix: bulk, formulations
Sample preparation: 100 mg Bulk drug or formulation containing 100-120 mg drug + 10 mL 1 (bulk) or 4 (formulations) mg/mL benzoic acid in MeOH:water 50:50 + 20 mL 5 mg/mL p-nitroacetophenone in MeOH, make up to 100 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:glacial acetic acid 20:5:74:1 containing 0.05-0.08% sodium 1-heptanesulfonate, pH 3.1
Flow rate: 2
Injection volume: 5
Detector: UV 278

CHROMATOGRAM
Retention time: 6
Internal standard: benzoic acid, p-nitroacetophenone (12)

OTHER SUBSTANCES
Simultaneous: 4-amino-2-chlorobenzoic acid, impurities, chloroprocaine

KEY WORDS
benzoic acid is IS

REFERENCE
Menon,G.; Norris,B.; Webster,J. Simultaneous determination of chloroprocaine hydrochloride and its degradation product 4-amino-2-chlorobenzoic acid in bulk drug and injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 251–253.

SAMPLE
Matrix: food
Sample preparation: 5 g Food + 2 mL 1 mg/mL 4-hydroxyacetanilide in MeOH, make up to 15 mL with MeOH, mix, centrifuge at 1500 g for 10 min, filter an aliquot of the supernatant (0.45 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: RP18 (Brownlee)

Column: 10 μm Spheri RP-18 (Brownlee)
Mobile phase: MeOH:30 mM pH 6.5 phosphate buffer 5:95
Flow rate: 2
Injection volume: 15
Detector: UV 227

CHROMATOGRAM
Retention time: 4.24
Internal standard: 4-hydroxyacetanilide (14.57)

OTHER SUBSTANCES
Simultaneous: sorbic acid

KEY WORDS
beverages; fruit; seafood; vegetables; sauces; dairy products

REFERENCE
Bui,L.V.; Cooper,C. Reverse-phase liquid chromatographic determination of benzoic and sorbic acids in foods, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 892–896.

SAMPLE
Matrix: food
Sample preparation: 2 g Soy sauce or 1 g sugared fruit or roast beef + 10 g NaCl, make up to 50 mL with acetone, swirl vigorously, let stand for 30 min, filter, wash the solid with acetone, make up filtrate to 50 mL with acetone, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm monomeric C18 (Shoko, Kyoto)
Mobile phase: MeCN:50 mM pH 4.5 α-hydroxyisobutyric acid in water 22:34 containing 2.5 mM hexadecyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM
Retention time: 24

OTHER SUBSTANCES
Simultaneous: acesulfame-K, 3-t-butyl-4-hydroxyanisole, butyl p-hydroxybenzoate, t-butylhydroxyquinone, dulcin, ethyl p-hydroxybenzoate, isobutyl p-hydroxybenzoate, isopropyl p-hydroxybenzoate, methyl p-hydroxybenzoate, saccharin, sodium dehydroacetate, sorbic acid

KEY WORDS
soy sauce; roast beef; sugared fruit

REFERENCE
Chen,B.H.; Fu,S.C. Comparison of extraction methods and column types for the determination of additives by liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 625–643.

SAMPLE
Matrix: formulations
Sample preparation: 5 mL Formulation + 5 mL IS solution, make up to 50 mL with MeOH, inject 10 μL aliquot. (IS solution was 0.2 mg p-nitroacetophenone and 2.5 mg isobutyrophenone per mL of MeOH.)

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water:reagent 25:60:15, pH 2.6 (Reagent was MeOH containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate.)
Flow rate: 2
Injection volume: 10
Detector: UV 257

CHROMATOGRAM
Retention time: 3.5
Internal standard: p-nitroacetophenone (6) and isobutyrophenone (13)

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, hydroxyzine, benzaldehyde, p-chlorobenzoic acid, p-chlorobenzaldehyde, p-chlorobenzophenone

KEY WORDS
injections; stability-indicating

REFERENCE
Menon,G.N.; Norris,B.J. Simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol in injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 697–698.

SAMPLE
Matrix: formulations
Sample preparation: Leach 200 or 300 mg ground capsule or tablet with water or mobile phase and dilute to 50 mL, sonicate for 5 min, centrifuge at 2500 rpm for 5 min, inject an aliquot. Dilute 4-25 mL of liquid formulations to 250 mL with water, inject an aliquot.

HPLC VARIABLES
Column: Partisil-10 C8
Mobile phase: MeOH:MeCN:water:PIC-B5 50:170:755:25 (PIC-B5 (Waters) is 200 mM sodium pentanesulfonate in glacial acetic acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 14.5

OTHER SUBSTANCES
Simultaneous: phenylephrine, phenylpropanolamine, guaifenesin, impurities, degradation products

KEY WORDS
tablets; capsules; liquid formulations; stability-indicating

REFERENCE
Schieffer,G.W.; Smith,W.O.; Lubey,G.S.; Newby,D.G. Determination of the structure of a synthetic impurity in guaifenesin: modification of a high-performance liquid chromatographic method for phenylephrine hydrochloride, phenylpropanolamine hydrochloride, guaifenesin, and sodium benzoate in dosage forms, *J.Pharm.Sci.*, **1984**, *73*, 1856–1858.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 20:80 (Buffer was 15 mM 1-butanesulfonic acid + 15 mM KH_2PO_4 + 2 mL/L triethylamine, pH adjusted to 4.8 ± 0.1 with dilute phosphoric acid.)
Column temperature: 50
Flow rate: 2
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Simultaneous: codeine, acetaminophen, p-aminophenol, codeine N-oxide, codeinone

KEY WORDS
elixir; stability-indicating

REFERENCE
Sisco,W.R.; Rittenhouse,C.T.; Everhart,L.A.; McLaughlin,A.M. Simultaneous high-performance liquid chromatographic stability-indicating analysis of acetaminophen, codeine phosphate, and sodium benzoate in elixirs, *J.Chromatogr.*, **1986**, *354*, 355–366.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 2 g metronidazole benzoate suspension containing 3.5% metronidazole benzoate, add 80 mL MeOH:water 80:20, sonicate for a few minutes, make up to 100 mL with MeOH water 80:20, centrifuge at 900 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 2 30-38 μm Whatman Co:Pell
Column: 250 × 4.6 10 μm Perkin-Elmer C18
Mobile phase: MeOH:water 60:40 containing 5 mM acetate buffer and 50 mM KNO_3, pc_H* 5.2
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: metronidazole, metronidazole benzoate, methylparaben, propylparaben

KEY WORDS
suspensions

REFERENCE
Pashankov,P.P.; Kostova,L.L. Reversed-phase high-performance liquid chromatography of metronidazole benzoate in suspension dosage form, *J.Chromatogr.*, **1987**, *394*, 382–387.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL syrup to 50 mL with mobile phase, filter (0.45 μm), inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:water:formic acid:methanesulfonic acid 500:500:1:1, pH adjusted to 3.5 with 10% NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: guaifenesin, saccharin, dextromethorphan

KEY WORDS
syrup

REFERENCE
Chen,T.M.; Pacifico,J.R.; Daly,R.E. High-pressure liquid chromatographic assay of dextromethorphan hydrobromide, guaifenesin, and sodium benzoate in an expectorant syrup, *J.Chromatogr.Sci.*, **1988**, *26*, 636–639.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out ground tablets or capsules equivalent to 150 mg bepridil.HCl, add 150 mL MeCN, shake for 30 min, dilute to 200 mL with MeCN, filter (Schleicher & Schüll paper, grade 588), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 580:405 (Buffer was 1.1 g sodium 1-heptanesulfonate in 405 mL water, adjust to pH 2.37 with glacial acetic acid (ca. 15 mL).)
Column temperature: 35
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Simultaneous: impurities, benzaldehyde, N-benzylaniline, bepridil

KEY WORDS
capsules; tablets

REFERENCE
Renzi,N.L.; Fronheiser,M.E.; Duong,H.T.; Fulton,D.J.; Rabinowitz,M. Stability-indicating high-performance liquid chromatography assay for bepridil hydrochloride drug substance and drug products, *J.Chromatogr.*, **1989**, *462*, 398–405.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: C18

Mobile phase: MeCN:buffer 13:87 (Buffer was 0.01% acetic acid containing 0.001% sodium octanesulfonate.)
Flow rate: 2.5
Detector: UV 280

CHROMATOGRAM
Internal standard: benzoic acid

OTHER SUBSTANCES
Simultaneous: dopamine

KEY WORDS
injections; 5% dextrose; benzoic acid is IS

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 10 mL to 1 L with water.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:diethylamine:glacial acetic acid 250:739:1:10, apparent pH 4.1
Column temperature: 35
Flow rate: 1.3
Injection volume: 25
Detector: UV 273

CHROMATOGRAM
Retention time: 5.3

OTHER SUBSTANCES
Simultaneous: guaifenesin, dextromethorphan

KEY WORDS
liquid formulations; stability-indicating

REFERENCE
Wilson,T.D.; Jump,W.G.; Neumann,W.C.; San Martin,T. Validation of improved methods for high-performance liquid chromatographic determination of phenylpropanolamine, dextromethorphan, guaifenesin and sodium benzoate in a cough-cold formulation, *J.Chromatogr.*, **1993**, *641*, 241–248.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 4 mL of 1 mg/mL solution and make up to 100 mL with mobile phase, inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax cyano special
Mobile phase: MeCN:buffer 50:50 (Buffer was 1% triethylamine, pH adjusted to 6 with conc phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: fluoxetine

KEY WORDS
syrup; elixir

REFERENCE
Peterson,J.A.; Risley,D.S.; Anderson,P.N.; Hostettler,K.F. Stability of fluoxetine hydrochloride in fluoxetine solution diluted with common pharmaceutical diluents, *Am.J.Hosp.Pharm.*, **1994**, *51*, 1342–1345.

SAMPLE
Matrix: juice
Sample preparation: Condition a Sep-Pak Classic SPE cartridge with 2 mL MeOH and 5 mL water. Centrifuge 10 mL orange juice at 1500 g for 5 min, add 1 mL supernatant to the SPE cartridge, wash with 3 mL hexane:MeCN 98:2, force air through cartridge three times, elute with 3 mL MeOH, make eluate up to 3 mL with MeOH, filter (0.45 μm)

HPLC VARIABLES
Guard column: PRP-1 (Hamilton)
Column: 250 × 4.1 10 μm PRP-1 (Hamilton)
Mobile phase: MeCN:buffer 40:60 (Buffer was 6.8 g KH_2PO_4 in 1 L water, adjust pH to 2.3 with 85% phosphoric acid.)
Flow rate: 0.7
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 7.26
Limit of detection: 0.5 ppm

KEY WORDS
orange; SPE

REFERENCE
Lee,H.S. Liquid chromatographic determination of benzoic acid in orange juice: Interlaboratory study, *J.AOAC Int.*, **1995**, *78*, 80–82.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: Lichrospher 100 RP-18
Mobile phase: MeOH:water containing 10 mM citric acid 45:55
Column temperature: 45
Flow rate: 1
Detector: UV 230

KEY WORDS
rat; intestine; Caco-2 monolayer

REFERENCE
Takagi,M.; Taki,Y.; Sakane,T.; Nadai,T.; Sezaki,H.; Oku,N.; Yamashita,S. A new interpretation of salicylic acid transport across the lipid bilayer: Implication of pH-dependent but not carrier-mediated absorption from the gastrointestinal tract, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1175–1180.

SAMPLE

Matrix: solutions

Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES

Column: 100 × 4 10 μm Radial-Pak C18 (Waters)

Mobile phase: Gradient. MeOH:water from 57:43 to 100:0 over 20 min, maintain at 100:0 for 12 min

Flow rate: 2

Injection volume: 5

Detector: F ex 370 em 450

CHROMATOGRAM

Retention time: 9.4

Limit of detection: 0.3-1 fmole

OTHER SUBSTANCES

Simultaneous: p-aminobenzoic acid, arachidic acid, arachidonic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, salicylic acid, stearic acid, thymidine, uridine, valeric acid

KEY WORDS

derivatization

REFERENCE

Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *346*, 227–236.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: ascorbic acid, salicylic acid, quinine, dihydroquinine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer:triethylamine 4:4:92:0.01 (Buffer was 0.05% sodium octanesulfonate adjusted to pH 2.2 with 3 M phosphoric acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 215

CHROMATOGRAM

Retention time: 2.4

OTHER SUBSTANCES

Simultaneous: p-aminobenzoic acid, benzaldehyde, benzyl alcohol, protirelin

REFERENCE

Rao,G.N.; Sutherland,J.W.; Menon,G.N. High-performance liquid chromatographic assay for thyrotropin releasing hormone and benzyl alcohol in injectable formulation, *Pharm.Res.*, **1987**, *4*, 38–41.

SAMPLE

Matrix: solutions
Sample preparation: React the carboxylic acid, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in MeCN at 45° for 2 h, inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for

12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.6 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5
Limit of detection: 1 pmole

OTHER SUBSTANCES
Simultaneous: quinoxaline-2-carboxylic acid, salicylic acid

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Inject 50 μL onto column A in series with column B, after 1.1 min switch so that column A comes after column B, continue to elute.

HPLC VARIABLES
Column: A 15 × 3.2 7 μm New Guard RP-18 (Applied Biosystems); B 100 × 4.6 3 μm Econosphere C18 (Alltech)
Mobile phase: MeCN:water:phosphoric acid 25:75:0.2
Flow rate: 1
Injection volume: 50
Detector: UV 200

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Extracted: toluene, cresol, phenol

KEY WORDS
groundwater; water; column-switching

REFERENCE

Chamkasem,N.; Hill,K.D.; Sewell,G.W. High-performance liquid chromatographic column-switching technique for the determination of intermediates of anaerobic degradation of toluene in ground water microcosm, *J.Chromatogr.*, **1991**, *587*, 185–191.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL aliquot of a 5-1000 nM solution of a carboxylic acid in MeCN with 100 μL 18-crown-6 solution, add 100 μL 100 μM dansyl-BAP in MeCN, mix, let stand at room temperature (aliphatic acids) or 55° (aromatic acids) for 30 min, add 100 μL 3 mM thymine in MeCN, add 5 mg potassium bicarbonate, vortex for 30 s, let stand for 30 min, evaporate to dryness under a stream of nitrogen. Reconstitute with dichloromethane, add to a Bond-Elut silica SPE cartridge, elute with 1.5 mL MeCN:dichloromethane 50:50. Evaporate the eluate to dryness and reconstitute the residue with 500 μL mobile phase, inject a 25 μL aliquot. (Prepare 18-crown-6 solution by sonicating a 1 mg/mL solution of 18-crown-6 in MeCN containing 1 mg/mL potassium bicarbonate for 20 min. Prepare dansyl-BAP (N-(bromoacetyl)-N'-[5-(dimethylamino)naphthalene-1-sulfonyl]piperazine) as follows. Slowly add a solution of 135 mg dansyl chloride in 30 mL acetone to a 10-fold molar excess of piperazine in acetone:water 75:25, stir at 50° for 1 h, evaporate the acetone. Acidify the remaining aqueous layer with concentrated nitric acid, wash 3 times with 15 mL portions of dichloromethane, adjust the pH of the aqueous layer to 11 with concentrated NaOH, extract three times with 15 mL portions of dichloromethane. Combine the extracts and dry them over anhydrous calcium chloride, concentrate to about 5 mL, chromatograph on a 400 × 25 column of 60-200 μm silica gel Si-60, wash with about 20 mL dichloromethane:MeOH 99:1 to remove a small fluorescent band, elute with about 30 mL dichloromethane:MeOH 95:5-94:6 to obtain a solution of dansylpiperazine, determine the concentration by UV absorption at 340 nm (extinction coefficient = 4300 in MeOH). Stir a solution of 700 mg bromoacetic acid and 1.1 g dicyclohexylcarbodiimide in 100 mL MeCN at room temperature for 1 min, slowly add 470 μmoles dansylpiperazine in MeCN, stir for 1 h, evaporate to dryness, reconstitute the residue with 10 mL dichloromethane, filter. Chromatograph the filtrate on a 400 × 25 column of 40-60 μm Si-60 silica gel with dichloromethane, when the first-eluting, strongly-fluorescent yellow band reaches the outlet change the eluent to dichloromethane:MeOH 99:1, collect about 50 mL eluate to obtain dansyl-BAP.)

HPLC VARIABLES

Column: 150 × 3.1 5 μm LiChrosorb RP-18

Mobile phase: MeCN:water 60:40, containing 2.5 mM pH 7.0 imidazole buffer

Flow rate: 0.5

Injection volume: 25

Detector: F ex 246 em 490 (cut-off filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN containing 5 mM bis(2-nitrophenyl)oxalate pumped at 0.3 mL/min and the mixture flowed immediately to the detector. (Prepare bis(2-nitrophenyl)oxalate by dissolving 13.9 g 2-nitrophenol in 250 mL benzene (Caution! Benzene is a carcinogen!), remove 50 mL benzene by azeotropic distillation, cool to 10°, add 10.1 g freshly distilled triethylamine, add 7 g oxalyl chloride dropwise, allow to warm to room temperature, let stand overnight, evaporate to dryness under reduced pressure, recrystallize to give bis(2-nitrophenyl)oxalate (J. Chem. Educ. 1974, 51, 529).)

CHROMATOGRAM

Retention time: 24

Limit of detection: 0.8-1 pmole

OTHER SUBSTANCES

Simultaneous: 2,4-dichlorobenzoic acid, ibuprofen, lipoic acid, 2-methoxybenzoic acid, naproxen, octanoic acid

KEY WORDS
derivatization; post-column reaction; SPE

REFERENCE
Kwakman,P.J.M.; Van Schaik,H.P.; Brinkman,U.A.T.; de Jong,G.J. *N*-(Bromoacetyl)-*N'*-[5-(dimethylamino)naphthalene-1-sulfonyl]piperazine as a sensitive labeling reagent for the determination of carboxylic acids by liquid chromatography with peroxyoxalate chemiluminescence and fluorescence detection, *Analyst*, **1991**, *116*, 1385–1391.

SAMPLE
Matrix: solutions
Sample preparation: Dilute a 10 mg/mL solution of caffeine in water 1:1000 with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 2540 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeOH:10 mM ammonium acetate and 2.5 mM sodium heptanesulfonate 20:80, pH adjusted to 5.1 with glacial acetic acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: caffeine

KEY WORDS
water

REFERENCE
Donnelly,R.F.; Tirona,R.G. Stability of citrated caffeine injectable solution in glass vials, *Am.J.Hosp.Pharm.*, **1994**, *51*, 512–514.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cor-

tisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.69

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Acidify 1 mL solution with 200 μL 100 mM HCl, extract with ether. Evaporate the ether to dryness, reconstitute with 3 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 Lichrosphere 100RP-18
Column: 250 × 4 Lichrosphere 100RP-18
Mobile phase: MeCN:10 mM pH 6.8 phosphate buffer containing 10 mM sec-butylamine 5:95
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 305 em 407

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: 4-aminosalicylic acid

REFERENCE
Motohashi,N.; Saito,Y. Rate constants for reaction of hydroxyl radicals with sulfapyridine and aminosalicylic acids, *Chem.Pharm.Bull.*, **1996**, *44*, 163–166.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve salt of pindolol in MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil RP-18
Mobile phase: Gradient. MeOH:buffer from 20:80 to 80:20 over 10 min. (Buffer was 2% acetic acid containing 1.1% sodium 1-heptanesulfonate.)
Column temperature: 40
Flow rate: 1.5
Detector: UV 273

CHROMATOGRAM
Retention time: 5.9

OTHER SUBSTANCES
Simultaneous: 2-methoxyphenylacetic acid (UV 270), pindolol (UV 254)

REFERENCE
Pietiläinen,H.; Saesmaa,T. HPLC determination of pindolol benzoate and pindolol 2-methoxyphenylacetate, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 583–591.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine at 2000 g for 10 min, filter (0.45 μm Millex-HV), dilute ten-fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:25 mM pH 4.5 acetate buffer 5:95
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 10.4
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: phenylacetic acid, hippuric acid, phenaceturic acid

KEY WORDS
sheep

REFERENCE
Arín,M.J.; Diez,M.T.; Resines,J.A. Rapid and simple method for the determination of urinary benzoic and phenylacetic acids and their glycine conjugates in ruminants by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *582*, 13–18.

SAMPLE
Matrix: vegetables
Sample preparation: Place 25 g homogenized vegetables in a flask and make up to 100 mL with MeOH:water 60:40, shake vigorously for 10 min, filter (paper). Centrifuge an aliquot of the filtrate at 11600 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 10 × 4 Spherisorb ODS-2
Column: 250 × 4 Spherisorb ODS-2
Mobile phase: 30 mM pH 6.7 Phosphate buffer (Buffer was 2.5 g $K_2HPO_4.3H_2O$ and 2.5 g KH_2PO_4 in 1 L water.)
Flow rate: 2
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 1 ppm

OTHER SUBSTANCES
Extracted: sorbic acid

KEY WORDS
peppers; tomatoes; caperberries; onions

REFERENCE

Montaño,A.; Sánchez,A.H.; Rejano,L. Determination of benzoic and sorbic acids in packaged vegetable products. Comparative evaluation of methods, *Analyst*, **1995**, *120*, 2483–2487.

SAMPLE

Matrix: wine

Sample preparation: Adjust pH of wine to 7-8 with potassium bicarbonate. Remove a 1 mL aliquot and add it to 1 mL 170 mM phenacyl bromide in acetone, add 1 mL 17 mM 18-crown-6 in acetone, add 1 mL acetone, heat in a boiling water bath for 75 min, cool, inject a 10 μL aliquot. (Recrystallize phenacyl bromide from n-heptane.)

HPLC VARIABLES

Guard column: 37-50 μm Bondapak C18/Corasil
Column: 250 × 4 7 μm RP-18 (Merck)
Mobile phase: Gradient. MeOH:water from 35:65 to 85:15 over 20 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 14.7

OTHER SUBSTANCES

Extracted: acetic acid, anisic acid, benzilic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, salicylic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid, ascorbic acid

KEY WORDS

derivatization

REFERENCE

Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

SAMPLE

Matrix: yogurt

Sample preparation: 500 mg Homogenized yogurt + 7 mL buffer, sonicate for 2 min, shake mechanically for 20 min, centrifuge at 2000 rpm for 10 min, repeat extraction twice more. Combine the supernatants and make up to 25 mL with buffer. Remove a 5 mL extract and add it to 5 mL 10 mM tri-n-octylamine in chloroform, shake for 20 min, centrifuge at 2000 rpm for 10 min. Remove 2.5 mL of the organic phase and add it to 2.5 mL 100 mM sodium perchlorate in water, extract, centrifuge, inject an aliquot of the aqueous phase. (Buffer was 24.650 g $NaH_2PO_4.H_2O$ and 1.260 g $Na_2HPO_4.2H_2O$ made up to 2 L with water, pH 5.5.)

HPLC VARIABLES

Column: 250 × 4 10 μm RP-18 (Merck)
Mobile phase: MeOH:buffer 40:60 (Buffer was 900 μL 1 M phosphoric acid and 27.598 g $NaH_2PO_4.H_2O$ made up to 2 L with water, pH 4.5.)
Injection volume: 100
Detector: UV 270 for 4 min then UV 240

CHROMATOGRAM

Retention time: 5

Limit of detection: 20 μg/g

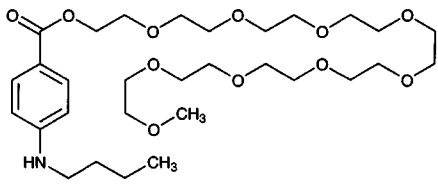
OTHER SUBSTANCES

Simultaneous: saccharin, sorbic acid

REFERENCE

Puttemans,M.L.; Branders,C.; Dryon,L.; Massart,D.L. Extraction of organic acids by ion-pair formation with tri-n-octylamine. Part 6. Determination of sorbic acid, benzoic acid, and saccharin in yogurt, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 80–82.

Benzonatate



Molecular formula: $C_{30}H_{53}NO_{11}$
Molecular weight: 603.75
CAS Registry No.: 104-31-4
Merck Index: 1127

SAMPLE

Matrix: tissue
Sample preparation: Homogenize 10 g tissue in 40 mL water, make alkaline with NaOH, extract twice with ether. Combine extracts and evaporate them to dryness, reconstitute the residue in 1 mL EtOH, inject a 50 µL aliquot.

HPLC VARIABLES

Column: Reverse phase ODS
Mobile phase: MeCN:100 mM phosphoric acid 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 205

CHROMATOGRAM

Limit of detection: 200 ng/mL

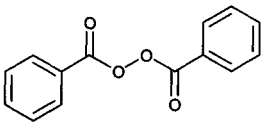
KEY WORDS

liver; brain; blood; kidney

REFERENCE

Cohan,J.A.; Manning,T.J.; Lukash,L.; Long,C.; Ziminski,K.R.; Conradi,S.E. Two fatalities resulting from Tessalon (benzonatate), *Vet.Hum.Toxicol.*, **1986**, *28*, 543–544.

Benzoyl peroxide



Molecular formula: $C_{14}H_{10}O_4$
Molecular weight: 242.23
CAS Registry No.: 94-36-0
Merck Index: 1149

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 mL MeCN and 2 mL 50 mM pH 7.5 phosphate buffer. Add 1 mL serum to the SPE cartridge, wash with 500 µL 50 mM pH 7.5 phosphate buffer, elute with 1 mL MeCN, inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

Column: Capcell Pak C-18 SG-120 (Shiseido)
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Injection volume: 20
Detector: UV 235

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: methyl methacrylate, N,N-dimethyl-p-toluidine

KEY WORDS

serum; dental material; methyl methacrylate polymer; plexiglass; horse; SPE

REFERENCE

Shintani,H.; Tsuchiya,T.; Hata,Y.; Nakamura,A. Solid phase extraction and HPLC analysis of toxic components eluted from methyl methacrylate dental materials, *J.Anal.Toxicol.*, **1993**, *17*, 73–78.

SAMPLE

Matrix: food
Sample preparation: Mix 1 g flour with 8.0 mL EtOH, shake vigorously for 1 min and extract by sonication for 15 min at room temperature. Centrifuge at 4000 rpm for 20 min, remove 4 mL supernatant, add 2 mL 100 mM KOH, mix in an ultrasonic water bath for 2 min at room temperature, dilute to 25 mL with water and set aside overnight. Filter (0.45 µm) and inject a 50 µL aliquot.

HPLC VARIABLES

Column: Dionex OmniPac PAX-100 anion exchange column
Mobile phase: MeOH:3 mM sodium carbonate 2:98
Flow rate: 1.0
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 19 ng/mL
Limit of quantitation: 120 ng/mL

OTHER SUBSTANCES
Interfering: benzoic acid

KEY WORDS
wheat flour; derivatization

REFERENCE
Chen,Q.-C.; Mou,S.-F.; Hou,X.-P.; Ni,Z.-M. Determination of benzoyl peroxide in wheat flour by ion chromatography with precolumn derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 705–716.

SAMPLE
Matrix: plastic
Sample preparation: Leach with 20 mL water, MeOH, acetone, or THF at room temperature for 24 h, repeat. Combine solutions and add an equal volume of MeCN:water 50:50, filter (0.45 μm) the supernatant, inject a 20 μl aliquot.

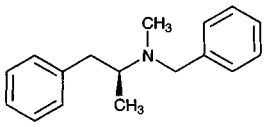
HPLC VARIABLES
Column: 250 × 4.6 Capcell Pak SG-120 C18 (Shiseido)
Mobile phase: MeCN:water 48:52
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 29.6

OTHER SUBSTANCES
Simultaneous: methyl methacrylate, N,N-dimethyl-p-toluidine

REFERENCE
Shintani,H. HPLC analysis of toxic additives and residual monomer from dental plate, *J.Liq.Chromatogr.*, **1995**, *18*, 613–626.

Benzphetamine



Molecular formula: $C_{17}H_{21}N$
Molecular weight: 239.36
CAS Registry No.: 156-08-1, 5411-22-3 (HCl)
Merck Index: 1151
Lednicer No.: 1 70

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 1.52

OTHER SUBSTANCES

Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

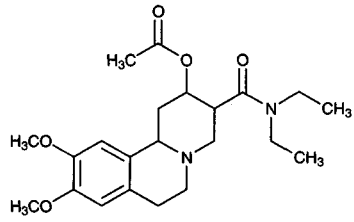
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Benzquinamide



Molecular formula: $C_{22}H_{32}N_2O_5$
Molecular weight: 404.51
CAS Registry No.: 63-12-7
Merck Index: 1154
Lednicer No.: 1 350

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.2

OTHER SUBSTANCES

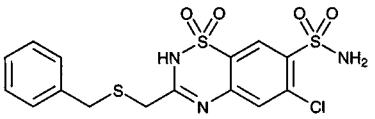
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Benzthiazide



Molecular formula: $C_{15}H_{14}ClN_3O_4S_3$
Molecular weight: 431.94
CAS Registry No.: 91-33-8
Merck Index: 1155

SAMPLE

Matrix: blood, feces, urine

Sample preparation: Plasma. 3 mL Plasma + 500 µL 1 µg/mL polythiazide in 10 mM NaOH + 1 mL 10 mM NaOH + 800 µL 100 mM HCl + 10 mL dichloromethane, shake on a platform shaker for 20 min, centrifuge at -10° at 3000 rpm for 15 min, repeat extraction twice more. Combine all organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 µL, vortex, inject whole amount. Urine. 5 mL Urine + 1 mL 2 µg/mL polythiazide in 10 mM NaOH + 1 mL 10 mM NaOH + 2 mL 0.68% KH_2PO_4 adjusted to pH 6.1 + 10 mL dichloromethane, shake on a platform shaker for 20 min, centrifuge at -10° at 3000 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 µL, vortex, inject a 20 µL aliquot. Feces. Extract 20 g feces with 350 mL acetone, filter (Whatman No. 1 paper), evaporate to ca. 50 mL, make up to 100 mL with acetone. Remove a 10 mL aliquot and add it to 1 mL 300 µg/mL polythiazide in acetone, evaporate to dryness under nitrogen, dissolve in 10 mL MeOH, inject a 10 µL aliquot.

HPLC VARIABLES

Column: µBondapak C18

Mobile phase: MeCN:glacial acetic acid:water 35:2:63 (plasma, feces) or MeCN:water 40:60 (urine)

Flow rate: 2

Injection volume: 10-50

Detector: UV 280

CHROMATOGRAM

Retention time: 7 (plasma), 5 (urine)

Internal standard: polythiazide (9 (plasma), 8 (urine))

Limit of detection: 10 ng/mL (plasma)

Limit of quantitation: 50 ng/mL (urine), 20 ng/mL (plasma)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Meyer,M.C.; Hwang,P.; Straughn,A.B.; Rotenberg,K. HPLC determination of benzthiazide in biologic material, *Biopharm.Drug Dispos.*, **1982**, *3*, 1–9.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: not specified

Mobile phase: MeOH:water containing 200 mM ammonia and 200 mM glycolic acid 25:75

Flow rate: 0.8

Detector: MS, Hewlett-Packard 9000-300 quadrupole, thermospray, stem 108-115°, tip 180-205°, ion source 276°, filament on, negative ion mode

KEY WORDS

LC-MS

REFERENCE

Kim,Y.; Park,S.; Park,J.; Lee,W. Detection of benzthiazide by high-performance liquid chromatography-thermospray mass spectrometry, *J.Chromatogr.A*, **1995**, *689*, 170–174.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 13.5 (A), 14.3 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 4.5:10.5:85:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES
Extracted: amiloride, chlorthalidone, triamterene, furosemide, bendroflumethiazide

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 17.6
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 6.5
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

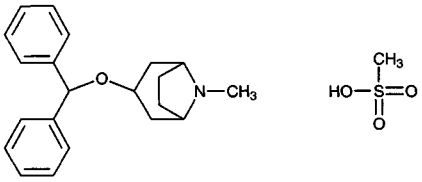
Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

Benztropine mesylate

Molecular formula: $C_{22}H_{29}NO_4S$
Molecular weight: 403.54
CAS Registry No.: 132-17-2, 86-13-5 (free base)
Merck Index: 1156



SAMPLE

Matrix: blood

Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 13.2
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 150 μL 200 ng/mL desipramine hydrochloride + 150 μL 5 M NaOH, vortex, add 1 mL ethylene glycol, vortex, add 10 mL hexane, shake on a rotary shaker at 30 rpm for 30 min, centrifuge at 1000 g at 4°. Remove the organic layer and add it to 300 μL 100 mM HCl, shake at high speed for 20 min, centrifuge, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Spherisorb C8

Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 199

CHROMATOGRAM

Retention time: 7.0
Internal standard: desipramine hydrochloride (5.3)
Limit of quantitation: 0.25 ng/mL

OTHER SUBSTANCES

Simultaneous: hyoscyamine, orphenadrine, bromocriptine, biperiden
Noninterfering: amantadine, carbidopa, levodopa

KEY WORDS

plasma

REFERENCE

Selinger,K.; Lebel,G.; Hill,H.M.; Discenza,C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma, *J.Chromatogr.*, **1989**, *491*, 248–252.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 22.75
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amoxapine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexiphenidyl, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: promethazine, trimipramine, amitriptyline

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.10

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

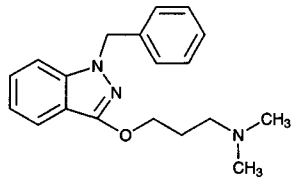
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenpro-

porex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Benzydamine



Molecular formula: $C_{19}H_{23}N_3O$
Molecular weight: 309.41
CAS Registry No.: 642-72-8, 132-69-4 (HCl)
Merck Index: 1157
Lednicer No.: 1 323

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 215.8

CHROMATOGRAM

Retention time: 14.955

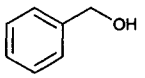
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Benzyl alcohol



Molecular formula: C_7H_8O
Molecular weight: 108.14
CAS Registry No.: 100-51-6
Merck Index: 1159

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 600 μg 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin in 2.5 mL toluene and 750 μL MeCN, add 20 μL benzyl alcohol, add 150 μL 93.3 μg/mL 4-dimethylaminopyridine in MeCN, mix vigorously, heat at 60° for 1.5 h, cool, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, inject a 5 μL aliquot. (Preparation of 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin is as follows. Stir 102.3 mg 7-(carboxymethoxy)-4-methylcoumarin in 7 mL THF, add 70.9 mg 1,1'-carbonyldiimidazole in one portion, reflux for 30 min, stir at room temperature for 5 h. Filter and dry the solid under reduced pressure to obtain 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin as a white solid (mp 161-162°). Fluorescence detection can also be used.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:100 mM pH 5.5 ammonium acetate buffer 50:1.5:48.5
Flow rate: 0.7
Injection volume: 5
Detector: MS, Hewlett-Packard 5989A, thermospray interface, filament-assisted ionization mode, ion source 280°, probe stem 112°, probe tip 235-245°

CHROMATOGRAM

Retention time: 7
Limit of detection: 0.8 ng

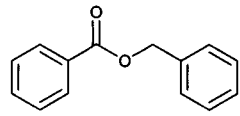
KEY WORDS

derivatization

REFERENCE

Phillips,L.R.; Supko,J.G.; Wolfe,T.L.; Malspeis,L. Precolumn derivatization of hydroxy compounds with 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin (IMMC) and LC/TSP-MS of the resulting esters, *Proc.Am.Soc.Mass Spectrom.*, **1995**, *43*, 163–164.

Benzyl benzoate



Molecular formula: $C_{14}H_{12}O_2$
Molecular weight: 212.25
CAS Registry No.: 120-51-4
Merck Index: 1162

SAMPLE

Matrix: formulations
Sample preparation: Dilute 0.5 mL of nanocapsules suspension 1:200 with MeCN, filter, inject an aliquot. Alternatively, evaporate 5 mL of a nanocapsule suspension to dryness and dissolve the residue in 150 mL dichloromethane or ethyl acetate, dry over anhydrous sodium sulfate. Evaporate to dryness under reduced pressure, take up the residue in 50 mL MeOH, dilute 1:20 with MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN: water 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.71

OTHER SUBSTANCES

Simultaneous: progesterone
Noninterfering: poly-ϵ-caprolactone

KEY WORDS

nanocapsules

REFERENCE

Benali,S.; Tharasse-Bloch,C.; André; Vérité,P.; Duclos,R.; Lafont,O. Determination od progesterone in nanocapsules by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3233–3243.

SAMPLE

Matrix: formulations
Sample preparation: Injections. Extract 2 mL with EtOH:water 85:15, make up extracts to 100 mL with EtOH:water 85:15, remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH:water 50:50, inject an aliquot. Suspensions. Dilute 2 mL suspension to 100 mL with EtOH, filter (if necessary), remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak CN
Mobile phase: MeOH:20 mM KH_2PO_4 30:70
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 6.5

Internal standard: hydrocortisone (2.5)

OTHER SUBSTANCES
Simultaneous: medroxyprogesterone acetate
Noninterfering: polyethylene glycol 4000, mystristyl-gamma-picolinium chloride, methylcellulose, thimerosal
Interfering: progesterone

REFERENCE
Das Gupta,V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 294–297.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 11.8
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), miborelone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 50 mg formulation, add 5 mL 1.5 mg/mL benzophenone in MeOH, make up to 50 mL with MeOH. Dilute 1 mL of this solution to 10 mL with MeOH, filter (0.45 μm PTFE membrane), inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 mm long Brownlee guard column

Column: 220 × 4.6 5 μm C18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: benzophenone (3.3)
Limit of quantitation: 7000 ng/mL

OTHER SUBSTANCES
Simultaneous: benzocaine

KEY WORDS
dermatological preparations

REFERENCE
Gigante,B.; Barros,A.M.V.; Teixeira,A.; Marcelo-Curto,M.J. Separation and simultaneous high-performance liquid chromatographic determination of benzocaine and benzyl benzoate in a pharmaceutical preparation, *J.Chromatogr.*, **1991**, *549*, 217–220.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 85:15
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Also analyzed: boldenone, testosterone, nandrolone, and their esters

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Zorbax BP-ODS
Mobile phase: MeCN:50 mM pH 7.2 sodium phosphate buffer 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

Internal standard: benzyl benzoate

OTHER SUBSTANCES

Extracted: HO-221

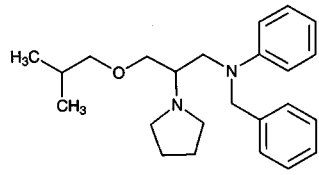
KEY WORDS

benzyl benzoate is IS

REFERENCE

Kondo,N.; Iwao,T.; Hirai,K.-I.; Fukuda,M.; Yamanouchi,K.; Yokoyama,K.; Miyaji,M.; Ishihara,Y.; Kon,K.; Ogawa,Y.; Mayumi,T. Improved oral absorption of enteric coprecipitates of a poorly soluble drug, *J.Pharm.Sci.*, **1994**, *83*, 566–570.

Bepridil



Molecular formula: $C_{24}H_{34}N_2O$
Molecular weight: 366.55
CAS Registry No.: 64706-54-3, 74764-40-2 (HCl monohydrate)
Merck Index: 1188
Lednicer No.: 3 46

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 249

CHROMATOGRAM

Retention time: 18.30
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 202.8

CHROMATOGRAM

Retention time: 19.503

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out ground tablets or capsules equivalent to 150 mg bepridil.HCl, add 150 mL MeCN, shake for 30 min, dilute to 200 mL with MeCN, filter (Schleicher & Schüll paper, grade 588), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 580:405 (Buffer was 1.1 g sodium 1-heptanesulfonate in 405 mL water, adjust to pH 2.37 with glacial acetic acid (ca. 15 mL).)
Column temperature: 35
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Simultaneous: impurities, benzaldehyde, N-benzylaniline, benzoic acid

KEY WORDS
stability-indicating; rugged; capsules; tablets

REFERENCE
Renzi,N.L.; Fronheiser,M.E.; Duong,H.T.; Fulton,D.J.; Rabinowitz,M. Stability-indicating high-performance liquid chromatography assay for bepridil hydrochloride drug substance and drug products, *J.Chromatogr.*, **1989**, *462*, 398–405.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 62:35:3 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 250

KEY WORDS
chiral; α = 1.22 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)

Mobile phase: EtOH:50 mM pH 5.5 KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: k' 11.96

OTHER SUBSTANCES

Simultaneous: lorazepam, manidipine, nicardipine, oxazepam

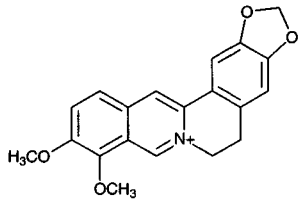
KEY WORDS

chiral; α = 1.21

REFERENCE

Massolini,G.; De Lorenzi,E.; Ponci,M.C.; Gandini,C.; Caccialanza,G.; Monaco,H.L. Egg yolk riboflavin binding protein as a new chiral stationary phase in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 55–65.

Berberine



Molecular formula: $C_{20}H_{18}NO_4$
Molecular weight: 336.37
CAS Registry No.: 2086-83-1
Merck Index: 1192

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

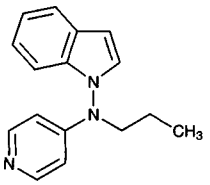
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, py-

rilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Besipirdine



Molecular formula: $C_{16}H_{17}N_3$
Molecular weight: 251.33
CAS Registry No.: 119257-34-0, 130953-69-4 (HCl), 119257-40-8 (maleate)
Merck Index: 1223

SAMPLE

Matrix: cell cultures
Sample preparation: Mix 1 mL cell culture with 50 μL 1 mg/mL IS in MeOH and 1 mL saturated aqueous sodium bicarbonate, add 3 mL ethyl acetate:cyclohexane 50:50, invert at 18 cycles/min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 2μm Hypersil-Phenyl
Mobile phase: MeCN:buffer 85:15 (Buffer was 5.8 mM triethylammonium formate adjusted to pH 2.5 with 90% formic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 10
Internal standard: 3-ethyl-N-methyl-N-(4-pyridinyl)-1H-indol-1-amine hydrochloride

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

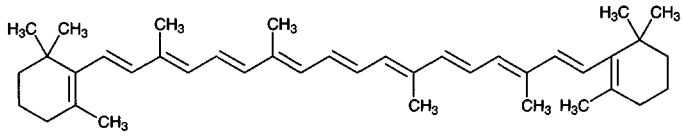
details of semipreparative HPLC

REFERENCE

Rao,G.P.; Davis,P.J. Microbial models of mammalian metabolism. Biotransformations of HP 749 (besperidine) using Cunninghamella elegans, *Drug Metab.Dispos.*, **1997**, *25*, 709–715.

Beta-carotene

Molecular formula: $C_{20}H_{56}$
Molecular weight: 536.88
CAS Registry No.: 7235-40-7
Merck Index: 1902



SAMPLE

Matrix: blood

Sample preparation: Centrifuge 200 µL serum, add 200 µL IS and 200 µL EtOH, mix on orbital shaker for 5 min, add 200 µL water and 500 µL hexane, mix for 10 min, centrifuge at 2000 g for 10 min at 17°, remove 300 µL upper organic layer. Re-extract with 300 µL hexane, mix for 10 min, centrifuge at 4000 g for 10 min at 17°, remove 300 µL upper organic layer. Combine the organic layers, and evaporate them to dryness under vacuum in 15 min. Reconstitute the residue with 300 µL MeOH:EtOH:hexane 88:10:2, vortex for 10 min, inject a 40 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 µm Adsorbosphere HS C18 + 150 × 4.6 3 µm Adsorbosphere HS C18 in series

Mobile phase: Gradient. A was MeCN:MeOH 60:40 containing 0.05% acetic acid. B was MeCN:MeCN:dichloromethane 45.6:30.4:24 containing 0.04% acetic acid. A:B 100:0 for 7 min then 0:100 for 10.4 min (step gradient), re-equilibrate at initial conditions for 5.6 min.

Column temperature: 37

Flow rate: 0.9

Injection volume: 40

Detector: UV 450

CHROMATOGRAM

Retention time: 16.5

Internal standard: tocol (UV 292) (10.1), echinenone (UV 450) (12.8)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: canthaxanthine (UV 473), α-carotene (UV 450), β-cryptoxanthine (UV 450), lutein (UV 450), lycopene (UV 473), vitamin A (UV 325), vitamin E (UV 292), zeaxanthin (UV 450), nonidentified carotenoids

KEY WORDS

serum

REFERENCE

Steghens,J.-P.; van Kappel,A.L.; Riboli,E.; Collombel,C. Simultaneous measurement of seven carotenoids, retinol and α-tocopherol in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *694*, 71–81.

SAMPLE

Matrix: blood

Sample preparation: Add 1 mL 1 µg/mL retinyl palmitate, 1 µg/mL retinyl palmitate, and 25 µg/mL α-tocopheryl acetate in EtOH to 1 mL serum or plasma while continuously vortexing, add 3 mL hexane, vortex for 2 min, centrifuge at 2500 g for 2 min, remove the upper phase, add 2 mL hexane to the lower layer, repeat extraction. Combine the upper layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL mobile phase, inject a 40 µL aliquot.

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 5 μm Biophase ODS C18 (Bioanalytical Systems)
Mobile phase: MeCN:chloroform:isopropanol:water 78:16:3.5:2.5
Flow rate: 2
Injection volume: 40
Detector: UV 460 (UV 292 for tocopherol)

CHROMATOGRAM
Retention time: 16.46
Internal standard: retinyl acetate (3.07), retinyl palmitate (18.66), α-tocopheryl acetate (8.33)

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), gamma-tocopherol, α-carotene, lycopene, cryptoxanthin

KEY WORDS
serum; plasma

REFERENCE
Kaplan,L.A.; Miller,J.A.; Stein,E.A.; Stampfer,M.J. Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma, *Methods Enzymol.*, **1990**, *189*, 155–167.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 25 μL 80 μg/mL tocol in EtOH + 250 μL 20 μg/mL BHT (butylated hydroxytoluene) in EtOH + 1.5 mL hexane, vortex for 1 min, remove 1 mL of upper layer, add 500 μL hexane, vortex for 1 min, remove 300 μL of upper layer. Combine the hexane extracts, evaporate to dryness under a stream of inert gas. Reconstitute in 250 μL 20 μg/mL BHT in EtOH, sonicate, centrifuge if necessary, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201TP54 (wide pore, polymerically bonded C18)
Mobile phase: Gradient. A was MeOH:n-butanol:water 75:10:15 containing 50 mM ammonium acetate, pH 5.5. B was MeOH:n-butanol:water 88:10:2 containing 50 mM ammonium acetate, pH 5.5. A:B 100:0 for 3 min, to 0:100 over 15 min, maintain at 0:100 for 17 min
Injection volume: 25
Detector: UV 325 for 7 min, UV 295 for 13 min, UV 450 for 14 min or E, glassy carbon electrode, Ag/AgCl reference electrode +1050 mV for retinol, +900 mV for tocol, +750 mV for α-tocopherol, +700 mV for β-carotene

CHROMATOGRAM
Retention time: 31
Internal standard: tocol (13)
Limit of detection: 2.1 μg/mL (E), 29 μg/mL (UV)

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), gamma-tocopherol, lutein, zeaxanthin, cryptoxanthin, α-carotene, 9-cis-β-carotene

KEY WORDS
serum

REFERENCE
MacCrehan,W.A. Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography, *Methods Enzymol.*, **1990**, *189*, 172–181.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL EtOH + 100 μL α-tocopheryl acetate in EtOH, vortex for 5 s, add 500 μL hexane, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 250 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, mix for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Spheri-10 RP18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:dichloromethane:MeOH 70:20:10
Flow rate: 1.2
Injection volume: 50
Detector: UV 325 for 3.5 min, UV 291 for 4.5 min, UV 450 for 6 min

CHROMATOGRAM
Retention time: 11.79
Internal standard: α-tocopheryl acetate (6.30)
Limit of detection: 50 nM

OTHER SUBSTANCES
Extracted: vitamin A, vitamin E

KEY WORDS
protect from light; serum

REFERENCE
Arnaud,J.; Fortis,I.; Blachier,S.; Kia,D.; Favier,A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 2.5 mL Plasma + 2.5 mL 18 ng/mL IS1 and 10 ng/mL IS2 in EtOH, shake vigorously for 20 s, centrifuge at 1200 g for 5 min, add 5 mL diethyl ether, shake vigorously, centrifuge for 5 min, extract twice more with 5 mL ether. Combine ether layers, wash with 15 mL 5% NaCl, dry over sodium sulfate, evaporate to dryness under vacuum at 35°. Dissolve residue in 1-2 mL dichloromethane, filter (0.45 μm). Evaporate to dryness under a stream of nitrogen, make up to 100 μL with MeCN:MeOH:dichloromethane:hexane 45:10:22.5:22.5, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spheri-5-C18 (Brownlee)
Column: 250 × 4.6 5 μm Microsorb C18 (Rainin)
Mobile phase: Gradient. MeCN:MeOH:dichloromethane:hexane 85:10:2.5:2.5 for 10 min then to 45:10:22.5:22.5 over 30 min, re-equilibrate for 15 min
Flow rate: 0.7
Injection volume: 20
Detector: UV 470

CHROMATOGRAM
Retention time: 34

Internal standard: IS1 ethyl β-apo-8'-carotenate (18), IS2 (3R)-8'-apo-β-carotene-3,8'-diol (5)

OTHER SUBSTANCES
Extracted: carotenoids, vitamin A (retinol), vitamin E (α-tocopherol)

KEY WORDS
plasma; handle under yellow lights

REFERENCE
Khachik,F.; Beecher,G.R.; Goli,M.B.; Lusby,W.R.; Smith,J.C.,Jr. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma, *Anal.Chem.*, **1992**, *64*, 2111–2122.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 25 μg/mL tocopheryl acetate in EtOH, vortex, add 400 μL butanol:ethyl acetate 50:50, mix for 1 min, add 20 mg sodium sulfate, vortex for 1 min, let stand at -20° for 20 min, centrifuge at 15000 g for 2 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES
Guard column: 5 μm C18
Column: 110 × 4.7 5 μm Partisphere 5 C18 (Whatman)
Mobile phase: MeOH:butanol:water 89.5:5:5.5
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: UV 340 for 3 min, UV 290 for 1.5 min, UV 280 for 10.5 min, UV 450 for 7 min

CHROMATOGRAM
Retention time: 20.1
Internal standard: tocopheryl acetate (5.3)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: α-carotene, lycopene, δ-tocopherol, gamma-tocopherol, vitamin A, vitamin E, xanthophyll

KEY WORDS
serum; plasma; protect from light

REFERENCE
Lee,B.L.; Chua,S.C.; Ong,H.Y.; Ong,C.N. High-performance liquid chromatographic method for routine determination of vitamins A and E and β-carotene in plasma, *J.Chromatogr.*, **1992**, *581*, 41–47.

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: MeCN:water:THF 81.3:5.7:13

Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 450

CHROMATOGRAM
Retention time: 14.230
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: Vitamin A (retinol), Vitamin E (α-tocopherol), probucol, gamma-tocopherol, lycopene, α-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 20-500 μL Serum + 2 volumes EtOH + 1 mL ethyl acetate + 4-7 μL of a solution containing 16 mg/mL tocopheryl acetate, 2-3 μg/mL canthaxanthin, and 10 μg/mL retinoic acid, vortex for 30 s, centrifuge for 30 s, extract the pellet twice with 0.5-1 mL portions of ethyl acetate, extract the pellet with 0.5-1 mL hexane. Combine the supernatants, add 500 μL water, vortex, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of argon, reconstitute the residue in 100 μL MeOH:dichloromethane 2:1, inject a 10-90 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:1-octanol 90:15:10:0.1
Flow rate: 1
Injection volume: 10-90
Detector: UV 450

CHROMATOGRAM
Retention time: 21
Internal standard: tocopheryl acetate, canthaxanthin, retinoic acid

OTHER SUBSTANCES
Extracted: carotenoids, vitamin A (UV 325), vitamin E (UV 290)

KEY WORDS
protect from light; serum

REFERENCE
Barua,A.B.; Kostic,D.; Olson,J.A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum, *J.Chromatogr.*, **1993**, *617*, 257–264.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 500 μL EtOH containing 4.27 μM retinyl acetate and 0.31 μM echinenone, rotamix for 30 s, add 2 mL n-hexane, rotamix for 30 s,

centrifuge at 2000 g for 2 min, repeat extraction with 2 mL n-hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL THF, make up to 200 μL with EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spherisorb ODS1
Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: Gradient. A was MeCN:MeOH 20:80 containing 100 mM ammonium acetate. B was 100 mM ammonium acetate in water. A:B from 90:10 to 100:0 over 12 min, maintain at 100:0 for 10 min, re-equilibrate at initial conditions for 5 min
Flow rate: 2
Injection volume: 50
Detector: UV 325 for 7.5 min, UV 292 for 5.5 min, then UV 450

CHROMATOGRAM
Retention time: 19.50
Internal standard: retinyl acetate (5.96), echinenone (15.15)
Limit of detection: 0.13 μM

OTHER SUBSTANCES
Extracted: cryptoxanthin, lutein, lycopene, vitamin A, vitamin E

KEY WORDS
plasma; protect from light; serum

REFERENCE
Zaman,Z.; Fielden,P.; Frost,P.G. Simultaneous determination of vitamins A and E and carotenoids in plasma by reversed-phase HPLC in elderly and younger subjects, *Clin.Chem.*, **1993**, *39*, 2229–2234.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL 850 ng/mL retinyl acetate in EtOH, mix for 1 min, add 1 mL 0.4 g/L BHT (2,6-di-tert-butyl-4-methylphenol) in n-hexane, shake on a mechanical shaker for 10 min, centrifuge at 2000 g for 5 min, remove 800 μL of the supernatant, evaporate to dryness at 40° under a stream of nitrogen, reconstitute in 100 μL MeCN:THF:MeOH 68:22:7, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Lichrosorb RP18
Column: 250 × 4.6 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:THF:MeOH 68:22:7 made up to 100 with 1% ammonium acetate
Flow rate: 1.5
Injection volume: 15
Detector: UV 325 for 3 min, UV 450 for 1.9 min, UV 290 for 2.5 min, UV 470 for 4.6 min, UV 450 for 3 min, then UV 325 for rest of run

CHROMATOGRAM
Retention time: 13
Internal standard: retinyl acetate (2.7)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), lutein, lycopene, α-carotene, zeaxanthin, trans β-carotene, δ-tocopherol

KEY WORDS
plasma; serum; protect from sunlight

REFERENCE
Bui,M.H. Simple determination of retinol, α-tocopherol and carotenoids (lutein, all-*trans*-lycopene, α- and β-carotenes) in human plasma by isocratic liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 129–133.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL nonapreno-β-carotene and retinyl butyrate in EtOH, vortex for 10 s, add 1 mL hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove 900 μL of the hexane layer and evaporate it to a waxy or glassy consistency (not dryness) under vacuum, dissolve in 100 μL EtOH, add 100 μL MeCN, vortex, filter (0.45 μm), inject a 30 μL aliquot.

HPLC VARIABLES
Column: 1540 × 4.6 5 μm Ultramex C18 (Phenomenex)
Mobile phase: MeCN:EtOH 50:50 containing 0.1 mL/L diethylamine
Column temperature: 29
Flow rate: 0.9
Injection volume: 30
Detector: UV 450

CHROMATOGRAM
Retention time: 8.10
Internal standard: nonapreno-β-carotene (9.5, UV 450), retinyl butyrate (3.5, UV 300)
Limit of detection: 13 nM

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, retinyl linoleate, retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
serum; use gold fluorescent lamps; hold sample at 4° before injection

REFERENCE
Sowell,A.L.; Huff,D.L.; Yeager,P.R.; Caudill,S.P.; Gunter,E.W. Retinol, α-tocopherol, lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection, *Clin.Chem.*, **1994**, *40*, 411–416.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 2.5 mL EtOH, mix for 5 min, add 5 mL n-hexane, mix vigorously, centrifuge at 2000 g for 5 min, repeat extraction with 3 mL n-hexane. Combine the n-hexane layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in dichloromethane, inject an aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak + 50 mm long C18 ODS(4) (Shimadzu)
Column: 250 × 4.6 5 μm Vydac 201 TP 54 C18
Mobile phase: MeOH:MeCN 90:10 (Every 100 injections wash column with MeOH:MeCN: dichloromethane 8:1:1.)
Flow rate: 1
Detector: UV 451

CHROMATOGRAM
Retention time: 18.5 (all-trans), 20.7 (9-cis), 21.8 (15-cis), 19.7 (9,15-dicis)

OTHER SUBSTANCES
Extracted: vitamin A (UV 324), vitamin E (UV 291)

KEY WORDS
serum

REFERENCE
Ben-Amotz,A. Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2813–2825.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 650 ng/mL tocopheryl acetate in MeOH, vortex for 30 s, add 200 μL n-hexane, shake for 15 min, centrifuge at 3000 rpm for 10 min. Remove 120 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 20 μL dichloromethane, add 100 μL MeCN:MeOH 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:dichloromethane 50:45:5
Flow rate: 0.7
Injection volume: 50
Detector: UV 450

CHROMATOGRAM
Retention time: 18.4 (all trans), 19.5 (15,15'-cis)
Internal standard: tocopheryl acetate (F ex 295 em 330) (9.3)

OTHER SUBSTANCES
Extracted: vitamin A (F ex 325 em 480), vitamin E (F ex 295 em 330), γ-tocopherol (F ex 295 em 330), retinyl palmitate (F ex 325 em 480), α-carotene

KEY WORDS
serum

REFERENCE
Yakushina,L.; Taranova,A. Rapid HPLC simultaneous determination of fat-soluble vitamins, including carotenoids, in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 715–718.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 0.9% NaCl + 200 μL MeOH, vortex for 30 s, let stand for 10 min, add 400 μL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot. Tissue. Homogenize (liver with Mikro-dismembrator II in liquid nitrogen; other tissue with Ultra-Turrax) tissue with 3 mL 1% acetic acid containing 1 mg/mL ascorbic acid and 10 mM EDTA, add 2 mL MeOH, vortex for 30 s, let stand for 10 min, add 4 mL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:dichloromethane:MeOH:water 70:10:15:5

Flow rate: 0.5 for 13 min, to 1 over 1 min, maintain at 1 for 10 min, to 1.5 over 1 min, maintain at 1.5 for 21 min, to 2 over 1 min, maintain at 2 for 10 min, return to 0.5 over 1 min, maintain at 0.5 for 2 min.
Injection volume: 50
Detector: UV 445

CHROMATOGRAM
Retention time: 39
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: vitamin A (UV 350), vitamin E (UV 292)

KEY WORDS
rat; protect from light; liver; plasma; lung

REFERENCE
Van Vliet,T.; Van Schaik,F.; Van Schoonhoven,J.; Schrijver,J. Determination of several retinoids, carotenoids and E vitamers by high-performance liquid chromatography. Application to plasma and tissues of rats fed a diet rich in either β-carotene or canthaxanthin, *J.Chromatogr.*, **1991**, *553*, 179–186.

SAMPLE
Matrix: cheese
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70° for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10. Combine the organic layers and evaporate them to dryness, dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 450 (β-carotene) and F ex 325 em 475 for 3.5 min, ex 280 em 475 for 10.5 min, ex 325 em 475 for 9 min (others)

CHROMATOGRAM
Retention time: 2
Limit of detection: 0.16 ng

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol), vitamin A (all-trans-retinol), β-tocopherol, gamma-tocopherol, δ-tocopherol, 13-cis-retinol

KEY WORDS
normal phase; cheese

REFERENCE
Panfili,G.; Manzi,P.; Pizzoferrato,L. High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses, *Analyst*, **1994**, *119*, 1161–1165.

SAMPLE
Matrix: food

Sample preparation: Dissolve 10 g margarine in 50 mL dichloromethane, add 3 g anhydrous magnesium sulfate, let stand for 2 h with frequent agitation, filter (fritted glass), make up filtrate to 100 mL with dichloromethane, inject a 250 μL aliquot on to four μStyragel 100 Å GPC columns (Waters) in series, elute with dichloromethane at 1 mL/min, monitor at 313 nm, collect the fraction corresponding to β-carotene (at 23.5-26.5 min) and evaporate it to dryness, reconstitute with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax C18
Mobile phase: MeCN:dichloromethane:MeOH 70:30:0.2
Flow rate: 1
Injection volume: 100
Detector: UV 436

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: vitamin A palmitate

KEY WORDS

margarine

REFERENCE

Chase,G.W.,Jr.; Akoh,C.C.; Eitenmiller,R.R.; Landen,W.O. Liquid chromatographic method for the concurrent analysis of sucrose polyester, vitamin A palmitate, and β-carotene in margarine, *J.Liq.Chromatogr.*, **1995**, *18*, 3129–3138.

SAMPLE

Matrix: fruit, juice, oil, vegetables
Sample preparation: Squash, peach. Homogenize (Waring blender) 10-30 g finely chopped squash or peach with 100 mL acetone and 10 g Hyflo supercel for 3 min, filter. repeat extraction of the solids until all the pigment was removed, extract with petroleum ether, wash the organic layer with water, pass it through a short column containing anhydrous sodium sulfate, concentrate under reduced pressure, inject an aliquot. Orange juice, palm tree oil. Homogenize (Waring blender) 10-30 g orange juice or palm tree oil with 100 mL acetone and 10 g Hyflo supercel for 3 min, filter. repeat extraction of the solids until all the pigment was removed, extract with petroleum ether, add 10% KOH in MeOH, let stand overnight, wash the organic layer with water, pass it through a short column containing anhydrous sodium sulfate, concentrate under reduced pressure, inject an aliquot.

HPLC VARIABLES

Column: 250 × 2.2 $Ca(OH)_2$ laboratory packed ($Ca(OH)_2$ from Nakarri Chemicals.)
Mobile phase: Isooctane (squash, palm tree oil) or Gradient. Isooctane:acetone from 100:0 to 80:20 over 1 h. (orange juice, peach)
Flow rate: 0.5
Injection volume: 10
Detector: UV 450

CHROMATOGRAM

Retention time: 10 (13-cis), 15 (trans), 22 (9-cis) (isocratic mobile phase)

KEY WORDS

squash; peach; orange juice; palm tree oil; normal phase

REFERENCE

Carvalho,C.R.L.; Carvalho,P.R.N.; Collins,C.H. High-performance liquid chromatographic determination of the geometrical isomers of β-carotene in several foodstuffs, *J.Chromatogr.A*, **1995**, *697*, 289–294.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 2-25 μL aliquot of a solution on mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C30 silica (Anal.Chem. 1994, 66, 1667)
Mobile phase: MTBE:MeOH 89:11
Flow rate: 1
Injection volume: 2-25
Detector: UV 453

CHROMATOGRAM
Retention time: 50 (all-trans)

OTHER SUBSTANCES
Simultaneous: isomers

REFERENCE
Emenhiser,C.; Sander,L.C.; Schwartz,S.J. Capability of a polymeric C_{30} stationary phase to resolve *cis-trans* carotenoid isomers in reversed-phase liquid chromatography, *J.Chromatogr.A*, **1995**, *707*, 205–216.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere-HS C18
Mobile phase: MeCN:isopropanol:MeOH 60:30:10 containing 0.1% ammonium acetate
Flow rate: 1
Detector: UV 234, UV 295, UV 450

OTHER SUBSTANCES
Simultaneous: vitamin E

REFERENCE
Maitra,I.; Marcocci,L.; Droy-Lefaix,M.T.; Packer,L. Peroxyl radical scavenging activity of *Gingko biloba* extract EGb 761, *Biochem.Pharmacol.*, **1995**, *49*, 1649–1655.

SAMPLE
Matrix: vegetables
Sample preparation: Macerate vegetables (Waring blender), remove 5 g of this material and homogenize it with 10 mL EtOH (Biohomogenizer) for 3 min, add 2 mL pentane, homogenize for 2 min (purge homogenizer motor with nitrogen), centrifuge at 7000 g for 3 min, remove pentane layer, add 2 g NaCl and 5 mL water to lower layer, shake gently, add 8 mL pentane, shake vigorously for 2 min, centrifuge, remove pentane layer. Combine pentane layers and evaporate (if necessary) under a stream of nitrogen to reduce the volume, make up to 5 or 10 mL with pentane, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201 TP54 C18
Mobile phase: MeOH:MeCN:dichloromethane:hexane 65:27:4:4
Flow rate: 1.5
Injection volume: 20
Detector: UV 450

CHROMATOGRAM
Retention time: 14

KEY WORDS
details for SFE also given; squash; broccoli; carrots; collard greens; turnip; kale; mustard greens; zucchini

REFERENCE
Marsili,R.; Callahan,D. Comparison of a liquid solvent extraction technique and supercritical fluid extraction for the determination of α- and β-carotene in vegetables, *J.Chromatogr.Sci.*, **1993**, *31*, 422–428.

SAMPLE
Matrix: vegetables
Sample preparation: Blanch vegetables in water for 90 s. Stir 20 g homogenized and mashed vegetables with 50 mL ethyl ether for about 4 h, filter, extract the residue again. Combine the filtrates, add 5 mL 20% KOH in MeOH, mix, place in a refrigerator for about 12 h, add 100 mL water, shake vigorously, let stand for about 5 h. Remove the ether layer and concentrate it under vacuum, evaporate traces of ether with a stream of nitrogen, reconstitute with EtOH (?), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Wakosil-II 5C18 AR polymeric ODS (Wako Chemicals)
Mobile phase: THF:MeOH 10:90
Column temperature: 20
Flow rate: 1
Injection volume: 100
Detector: UV 450

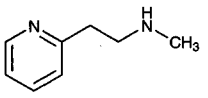
CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: α-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin

KEY WORDS
carrots; tomatoes; pumpkins

REFERENCE
Jinno,K.; Lin,Y. Separation of carotenoids by high-performance liquid chromatography with polymeric and monomeric octadecylsilica stationary phases, *Chromatographia*, **1995**, *41*, 311–317.

Betahistine



Molecular formula: $C_8H_{12}N_2$
Molecular weight: 136.20
CAS Registry No.: 5638-76-6, 5579-84-0 (2HCL)
Merck Index: 1224
Lednicer No.: 2 279

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.155

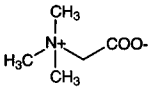
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Betaine



Molecular formula: $C_5H_{11}NO_2$

Molecular weight: 117.15

CAS Registry No.: 107-43-7, 590-46-5 (HCl), 590-47-6 (monohydrate), 93227-64-6 (sodium aspartate)

Merck Index: 1225

SAMPLE

Matrix: solutions

Sample preparation: Mix 600 µL solution with 60 µL IS solution, vortex, add a 550 µL aliquot to a 70 × 5 column of Dowex 1-X8 (OH^-) form in a Pasteur pipette, elute with 2 mL water, evaporate the eluate to dryness under a stream of air, reconstitute with 100 µL 2 mM N,N-diisopropylethylamine in MeCN, add 100 µL 5 mM 4'-bromophenacyl trifluoromethanesulfonate in MecN, vortex for 10 min, add 100 µL 10 mM hydroxyacetic acid N,N-diisopropylethylammonium salt in MeCN, vortex for 2 min, inject a 15 min aliquot. (Prepare 4'-bromophenacyl trifluoromethanesulfonate as follows. Add 8.8 g p-bromobenzoyl chloride in 40 mL dry ether over 20-30 min to 100 mmoles diazomethane stirred in an ice bath, stir in an ice bath for 8-9 h, let stand at room temperature for 3 h, evaporate the solvent under reduced pressure, recrystallize 4'-bromo-2-diazoacetophenone from ether/hexane (mp 123.5-124° d) (J.Am.Chem.Soc. 1951, 73, 5301). Condense 50 mL anhydrous sulfur dioxide in a flask fitted with a calcium sulfate drying tube, cool in a dry ice/acetone bath, add 2.25 g 4'-bromo-2-diazoacetophenone, stir for 5 min, add 900 µL anhydrous trifluoromethanesulfonic acid from a freshly opened bottle in one portion, stir for 15 min, remove the cooling bath, after 30 min use an ice/water bath to evaporate the solvent. Dissolve the residue in 100 mL boiling dichloromethane, treat twice with 5 g portions of decolorizing carbon, filter, evaporate the filtrate, recrystallize the residue from pentane:dichloromethane 80:20 to give 4'-bromophenacyl trifluoromethanesulfonate as colorless plates (mp 137-8°) (J. Chromatogr. 1984, 299, 365).)

HPLC VARIABLES

Guard column: 50 × 4 Co:Pell ODS

Column: 100 × 8 5 µm Radial-PAK C18

Mobile phase: MeCN:buffer 70:30 (Prepare by dissolving 70 mg sodium dodecyl sulfate, 140 mg $NaH_2PO_4.H_2O$, and 300 µL 3-dimethylamino-1-propanol in 150 mL water, adjusting pH to 6.5 with 85% phosphoric acid, and adding 350 mL MeCN.)

Flow rate: 5

Injection volume: 15

Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: (4-bromophenyl)carboxymethyl (6-trimethylammonium)hexanoate (Add an aqueous solution of 6-(trimethylammonium)hexanoic acid (Cl^- salt, prepared by methylation of 6-aminohexanoic acid) to a column of Dowex 1-X8 (OH^- form), elute with 4 column volumes of water. Evaporate the eluate to dryness, dissolve the residue in DMF, add 1.1 equivalents of triethylamine, add 1.1 equivalents of 2,4'-dibromoacetophenone, stir at 40° for 3 h, add ethyl acetate. Collect the precipitate by filtration and recrystallize it from ethanol/acetone to obtain (4-bromophenyl)carboxymethyl (6-trimethylammonium)hexanoate.) (8)

Limit of quantitation: 10 µM

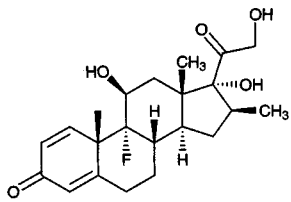
KEY WORDS

derivatization

REFERENCE

Minkler,P.E.; Ingalls,S.T.; Kormos,L.S.; Weir,D.E.; Hoppel,C.L. Determination of carnitine, butyrobetaine, and betaine as 4'-bromophenacyl ester derivatives by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *336*, 271–283.

Betamethasone



Molecular formula: $C_{22}H_{29}FO_5$

Molecular weight: 392.47

CAS Registry No.: 378-44-9, 987-24-6 (acetate), 22298-29-9 (benzoate), 5593-20-4 (dipropionate), 151-73-5 (sodium phosphate), 2152-44-5 (17-valerate), 5534-05-4 (acibutate), 360-63-4 (dihydrogen phosphate)

Merck Index: 1226

Lednicer No.: 1 198

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.277

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Condition a 500 mg diol SPE cartridge (Analytichem) with 6 mL dichloromethane. Sonicate a sample of cream containing 366 µg betamethasone valerate with 5 mL hexane:dichloromethane 70:30 for 5 min, make up to 10 mL with hexane:dichloromethane 70:30, add a 5 mL aliquot to the SPE cartridge, wash with 1 mL hexane:dichloromethane 70:30, elute with two 1 mL portions of MeOH, add 500 µL 180 µg/mL hydroxyresorcinol to the eluate, inject a 5 µL aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: MeOH:water 75:25
Flow rate: 0.5
Injection volume: 5
Detector: UV 235

CHROMATOGRAM
Retention time: 10 (betamethasone valerate)
Internal standard: hydroxyresorcinol

OTHER SUBSTANCES
Simultaneous: chlorocresol

KEY WORDS
cream; SPE

REFERENCE
Di Pietra,A.M.; Andrisano,V.; Gotti,R.; Cavrini,V. On-line post-column photochemical derivatization in liquid chromatographic-diode-array detection analysis of binary drug mixtures, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1191–1199.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.2
Detector: UV 242

CHROMATOGRAM
Retention time: 5 (betamethasone 17-valerate)

REFERENCE
Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

SAMPLE
Matrix: solutions
Sample preparation: Pass 20 mL of a solution in water (?) through an Empore C18 SPE disc. Wash with 2.5 mL water, dry, add 1 mL MeOH, let soak for 3 min, elute. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 1 mL running buffer, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb S5 ODS2
Mobile phase: MeOH:water 75:25
Flow rate: 1
Detector: UV 254

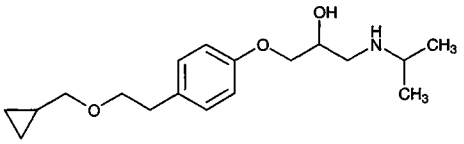
OTHER SUBSTANCES
Noninterfering: excipients

KEY WORDS
comparison with capillary electrophoresis; SPE

REFERENCE

Lucangioli,S.E.; Rodriguez,V.G.; Fernandez Otero,G.C.; Vizioli,N.M.; Carducci,C.N. Development and validation of capillary electrophoresis methods for pharmaceutical dissolution assays, *J.Capillary Electrophor.*, **1997**, *4*, 27–31.

Betaxolol



Molecular formula: $C_{18}H_{29}NO_3$
Molecular weight: 307.43
CAS Registry No.: 63659-18-7, 63659-19-8 (HCl)
Merck Index: 1229
Lednicer No.: 4 26

SAMPLE

Matrix: blood
Sample preparation: Add 5 mL chloroform:2-propanol:n-heptane 60:14:26, 1.5 mL saturated pH 9.5 ammonium chloride, and 40 μL 100 μg/mL prazepam to 2.0 mL postmorten blood. Shake horizontally for 20 min, centrifuge at 2800 g for 15 min. Evaporate organic phase under reduced pressure at 45°, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:tetrahydrofuran:100 mM pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Retention time: 4.32
Internal standard: prazepan (8.81)
Limit of quantitation: 100 ng/mL

KEY WORDS

whole blood

REFERENCE

Berthault,F.; Kintz,P.; Tracqui,A.; Mangin,P. A fatal case of betaxolol poisoning, *J.Anal.Toxicol.*, **1997**, *21*, 228–231.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cyanopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 300 μL MeOH:2-aminoheptane 99.7:0.3 at 1.5 mL/min, pass 700 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeCN:buffer 30:70 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37

Flow rate: 1.2
Injection volume: 250
Detector: F ex 230 em 300

CHROMATOGRAM
Retention time: 15

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 mM pH 7.4 phosphate buffer + 500 μL 2% zinc sulfate in MeOH:water 50:50, mix, centrifuge at 13000 rpm for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 SynChropak bulk support (Knauer)
Column: 120 × 4.6 5 μm Spherisorb ODS1 C18
Mobile phase: MeCN:MeOH:pH 4.5 acetate buffer (ratio not given)
Flow rate: 1
Detector: UV 222

CHROMATOGRAM
Retention time: 5.02

OTHER SUBSTANCES
Extracted: cyclopropane carboxylic acid ester prodrug

KEY WORDS
plasma

REFERENCE
Hovgaard,L.; Brondsted,H.; Buur,A.; Bundgaard,H. Drug delivery studies in Caco-2 monolayers. Synthesis, hydrolysis, and transport of O-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents, *Pharm.Res.*, **1995**, *12*, 387–392.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 223

CHROMATOGRAM

Retention time: 7.38
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Blood or urine + 100 μL 200 ng/mL (blood) or 90 μL 1 μg/mL (urine) cicloprolol hydrochloride in water + 1 mL water + 200 μL 1 M NaOH, vortex, add 10 mL diethyl ether, shake on a mechanical shaker for 30 min, centrifuge at 1400 g for 15 min. Remove the organic layer and add it to 2 mL 50 mM HCl, mix for 10 min. Remove

the aqueous phase and add it to 200 μL 1 M NaOH, add 10 mL diethyl ether, mix for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40-50°, reconstitute the residue in 100 μL 5 mM N,N-dimethyloctylamine, inject a 90 μL (blood) or 20-90 μL (urine) aliquot.

HPLC VARIABLES

Column: 75 × 4.5 3 μm Ultrasphere C18 (blood) or 150 × 4.5 5 μm Ultrasphere C18 (urine)
Mobile phase: MeCN:50 mM pH 3.0 N,N-dimethyloctylamine:water 8:10:82
Flow rate: 1
Injection volume: 20-90
Detector: F ex 200 no. 280 emission filter (Schoeffel Model FS 970)

CHROMATOGRAM

Retention time: 9 (blood), 29 (urine)
Internal standard: cicloprolol hydrochloride (6 (blood), 20 (urine))
Limit of detection: 10 ng/mL (urine), 1 ng/mL (blood)

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Wong,Y.W.J.; Ludden,T.M. Determination of βxolol and its metabolites in blood and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1990**, *534*, 161–172.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.405

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.3

OTHER SUBSTANCES
Also analyzed: alprenolol, bopindolol, propranolol, tertatolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.52, 1.57 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 9.86

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in 0.5% orthophosphoric acid.

HPLC VARIABLES

Column: 5 mm i.d. 4 μm Nova-Pak phenyl radial-Pak
Mobile phase: MeCN:MeOH:0.05% orthophosphoric acid 24:10:66
Flow rate: 1.6
Detector: F ex 261 em 306

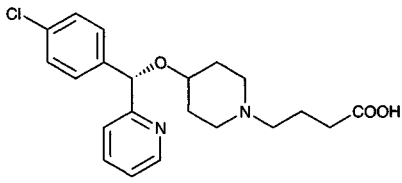
OTHER SUBSTANCES

Simultaneous: dextromethorphan, levorphanol (dextrorphan)

REFERENCE

Laslett,T.J.; Alvarez,F.; Nation,R.L.; Evans,A.M.; Scott,S.D.; Stupans,I. Effect of cyclophosphamide administration on the activity and relative content of hepatic P4502D1 in rat, *Xenobiotica*, **1995**, *25*, 1031–1039.

Betotastine



Molecular formula: $C_{21}H_{25}ClN_2O_3$
Molecular weight: 388.89
CAS Registry No.: 125602-71-3

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 9.0 phosphate buffer and 100 μL water, add 8 mL chloroform and extract. (Caution! Chloroform is a carcinogen!) Evaporate the organic layer, dissolve the residue in 400 μL mobile phase. Inject 200 μL aliquot. Tissue. Homogenize the brain with 2-fold the weight of water. Mix 1500 μL brain homogenate with 8 mL MeCN, centrifuge, evaporate 8 mL supernatant, dissolve the residue in 1 mL MeCN:water 50:50 and clean it up by a 1 mL 100 Bond Elute C18 SPE cartridge. Evaporate the effluent to dryness, dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES

Column: 250 × 6 Intersil ODS-2
Mobile phase: MeCN:0.018% TFA 17:83 (plasma), MeCN:0.018% TFA 18:82 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 220

CHROMATOGRAM

Limit of quantitation: 10 ng/mL (plasma), 35 ng/mL (brain)

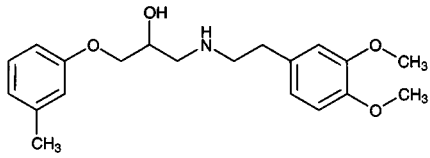
KEY WORDS

brain; cat; mouse; pharmacokinetics; plasma; rat; SPE

REFERENCE

Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

Bevantolol



Molecular formula: $C_{20}H_{27}NO_4$
Molecular weight: 345.44
CAS Registry No.: 59170-23-9, 42864-78-8 (HCl)
Merck Index: 1238
Lednicer No.: 3 28

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond-Elut C18 SPE cartridge with 1 volume MeCN and 1 volume 25 mM ammonium hydroxide, do not allow to dry. 500 µL Plasma + 80 µL water + 1 mL MeCN, vortex for 10 s, centrifuge at 2000 rpm for 10 min, remove the supernatant and add it to 1 mL 25 mM pH 10.5 ammonium hydroxide, vortex, add 100 µL 250 µg/mL 2,3,4,5-tetra-O-acetyl-α-D-glucopyranosyl isothiocyanate in MeCN (prepare fresh daily) with vortexing, let stand at room temperature for 10 min, add to SPE cartridge, wash with 1 volume 25 mM ammonium hydroxide, wash with 1 volume water, elute with 1.25 mL MeOH. Evaporate the eluent to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 µL mobile phase, inject a 75 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 µm Partisil 5 RAC II
Mobile phase: MeCN:75 mM $(NH_4)_2HPO_4$ adjusted to pH 3.5 with phosphoric acid 50:50
Column temperature: 45
Flow rate: 2
Injection volume: 75
Detector: UV 220

CHROMATOGRAM

Retention time: 6 (+), 7 (-)
Limit of detection: 20 ng/mL
Limit of quantitation: 40 ng/mL

KEY WORDS

plasma; chiral; SPE; derivatization

REFERENCE

Rose,S.E.; Randinitis,E.J. A high-performance liquid chromatographic assay for the enantiomers of bevantolol in human plasma, *Pharm.Res.*, **1991**, *8*, 758–762.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL pH 9.5 sodium bicarbonate buffer + 100 ng pronethalol, extract with 5 mL diethyl ether:dichloromethane 60:40. Remove the organic layer and extract it with 200 µL 100 mM HCl, inject the aqueous phase. Urine. Incubate urine with pH 5.5 acetate buffer and β-glucuronidase at 35°, buffer with 1 M pH 9.5 sodium carbonate, extract with diethyl ether:dichloromethane 60:40. Remove the organic layer and extract it with 100 mM HCl, inject the aqueous phase.

HPLC VARIABLES

Column: 5 µm Spherisorb ODS-2
Mobile phase: MeCN:MeOH:500 mM pH 3.5 phosphate buffer 30:20:50
Injection volume: 200
Detector: F (wavelengths not specified)

CHROMATOGRAM
Retention time: 3.0
Internal standard: pronethalol (plasma), verapamil (urine)
Limit of detection: 1000 ng/mL (urine), 50 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Nattel,S.; Lawand,S.; Matthews,C.; McCans,J. Bevantolol disposition in patients with hepatic cirrhosis, *J.Clin.Pharmacol.*, **1987**, *27*, 962–966.

SAMPLE
Matrix: feed
Sample preparation: 2 g Feed + 20 mL MeOH, rotate at 20 rpm for 1 h, centrifuge at 1300 rpm for 15 min, inject an aliquot.

HPLC VARIABLES
Guard column: 100 × 6.3 30-38 μm Co:Pell ODS (Whatman)
Column: 250 × 4.6 10 μm Lichrosorb RP-18
Mobile phase: MeOH:water:acetic acid 60:40:1 containing 5 mM sodium octanesulfonate (flush column with MeCN:water 50:50 after use)
Flow rate: 2
Injection volume: 50
Detector: UV 278

CHROMATOGRAM
Retention time: 10
Limit of detection: 50 μg/g

KEY WORDS
complexation

REFERENCE
Spurlock,C.H.; Schneider,H.G. Liquid chromatographic and ultraviolet spectrophotometric determination of bevantolol and hydrochlorothiazide in feeds, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 321–324.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

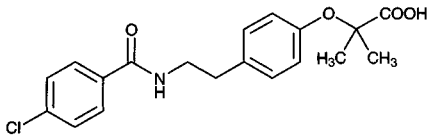
HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 10:90:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.97, 4.10 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

Bezafibrate



Molecular formula: $C_{19}H_{20}ClNO_4$
Molecular weight: 361.82
CAS Registry No.: 41859-67-0
Merck Index: 1240
Lednicer No.: 3 44

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.268

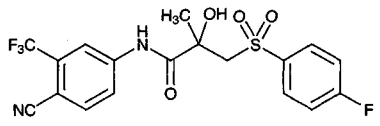
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bicalutamide



Molecular formula: $C_{18}H_{14}F_4N_2O_4S$
Molecular weight: 430.38
CAS Registry No.: 90357-06-5
Merck Index: 1247

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma, 1 mL 50 mM pH 7.0 phosphate buffer, 6 mL ethyl acetate, and 50 μL IS in MeOH, centrifuge. Remove a 5 mL portion of the organic layer and evaporate it to dryness. Reconstitute the residue in 400 μL MeCN:water 30:70, inject a 200 μL aliquot onto column A, elute with mobile phase A. Collect effluent containing the undifferentiated enantiomers, evaporate to dryness, reconstitute the residue in 400 μL MeCN:20 mM pH 7.0 phosphate buffer15:80. Inject a 200 μL aliquot onto column B. Elute with mobile phase B.

HPLC VARIABLES

Column: A 100 × 4.6 5 μm Hypersil ODS; B 5 μm AGP + 150 × 4.6 Ultron ES-OVM
Mobile phase: A MeCN:water 30:70; B MeCN:20 mM pH 7.0 phosphate buffer15:80
Injection volume: 200
Detector: UV

CHROMATOGRAM

Limit of detection: 5 ng/mL (R), 3.8 ng/mL (S)

KEY WORDS

plasma; pharmacokinetics; chiral

REFERENCE

Cockshott,I.D.; Oliver,S.D.; Young,J.J.; Cooper,K.J.; Jones,D.C. The effect of food on the pharmacokinetics of the bicalutamide ('casodex') enantiomers, *Biopharm.Drug Dispos.*, **1997**, *18*, 499–507.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize tissue in water. Buffer 500 μL intestinal homogenate with 1.5 mL 50 mM pH 7 phosphate buffer, add 6 mL ethyl acetate, mix, centrifuge. Remove a 5 mL portion of the organic layer and evaporate it to dryness, reconstitute the residue with 500 μL MeCN:water 50:50. Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:water 30:70
Injection volume: 50
Detector: UV

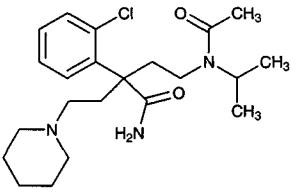
KEY WORDS

rat; jejunum; ileum; colon

REFERENCE

Cockshott,I.D.; Oliver,S.D.; Young,J.J.; Cooper,K.J.; Jones,D.C. The effect of food on the pharmacokinetics of the bicalutamide ('casodex') enantiomers, *Biopharm.Drug Dispos.*, **1997**, *18*, 499–507.

Bidisomide



Molecular formula: $C_{22}H_{34}ClN_3O_2$
Molecular weight: 407.98
CAS Registry No.: 103810-45-3
Merck Index: 1251

SAMPLE

Matrix: blood
Sample preparation: Alkalinize plasma with 1 M sodium hydroxide and extract with 1 mL chloroform (Caution! Chloroform is a carcinogen !). Centrifuge, transfer organic layer into tube and evaporate to dryness under a stream of dry nitrogen. Reconstitute samples with 200 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: Brownlee 5 µm CN
Mobile phase: MeCN:20 mM pH 4.0 monosodium phosphate 50:50
Flow rate: 1.0
Injection volume: 50
Detector: UV 207

CHROMATOGRAM

Internal standard: disopyramide
Limit of detection: 50 ng/mL

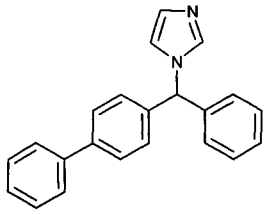
KEY WORDS

plasma; human; dog; pharmacokinetics

REFERENCE

Pao,L.-H.; Zhou,S.Y.; Cook,C.; Kararli,T.; Kirchhoff,C.; Truelove,J.; Karim,A.; Fleisher,D. Reduced systemic availability of an antiarrhythmic drug, bidisomide, with meal co-administration: Relationship with region-dependent intestinal absorption, *Pharm.Res.*, **1998**, *15*, 221–227.

Bifonazole



Molecular formula: $C_{22}H_{18}N_2$
Molecular weight: 310.40
CAS Registry No.: 60628-96-8
Merck Index: 1260
Lednicer No.: 4 93

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 1 mL MeOH, elute with 3 mL mobile phase. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: THF:buffer 30:70 (Buffer was 50 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 11
Internal standard: ketoconazole (7)

OTHER SUBSTANCES

Simultaneous: clotrimazole, ketoconazole, fenticonazole, tioconazole, isoconazole, econazole, miconazole

KEY WORDS

tablets; creams

REFERENCE

Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 85:15
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.6

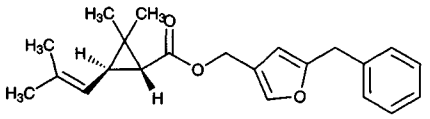
KEY WORDS

narrow-bore; chiral; α 1.56

REFERENCE

Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

Bioresmethrin



Molecular formula: $C_{22}H_{26}O_3$
Molecular weight: 338.45
CAS Registry No.: 28434-01-7
Merck Index: 1271

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm silica (IBM)
Mobile phase: Hexane:dichloromethane:isopropanol 99:1:0.07
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 6.56 (cis), k' 6.95 (trans)

OTHER SUBSTANCES
Also analyzed: allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, cyfluthrin (baythroid), permethrin, phenothrin, RU-11679, tetramethrin

KEY WORDS
normal phase

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

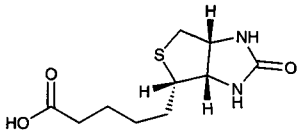
HPLC VARIABLES
Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.95:0.05
Flow rate: 0.8
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: phenothrin, permethrin

KEY WORDS
chiral

REFERENCE
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

Biotin



Molecular formula: $C_{10}H_{16}N_2O_3S$
Molecular weight: 244.31
CAS Registry No.: 58-85-5
Merck Index: 1272

SAMPLE

Matrix: blood, formulations

Sample preparation: Serum. Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 5 mL 1% acetic acid in water. 1 mL Serum + 1 mL 10% trichloroacetic acid, mix, centrifuge at 2000 g for 5 min, add a 1.5 mL aliquot of the supernatant to the SPE cartridge, wash with 10 mL 1% acetic acid in water, wash with 1 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness, reconstitute with 100 µL MeOH, sonicate, add 100 µL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, heat at 40° for 1 h, cool to room temperature, add 300 µL MeOH, inject a 10 µL aliquot. Tablets. Powder tablets, weigh out amount containing 500 µg biotin, add 30 mL MeOH, sonicate for 10 min, shake for 10 min, centrifuge at 3000 rpm for 5 min, remove the supernatant, repeat the extraction three times. Combine the supernatants and make up to 100 mL with MeOH. Remove a 200 µL aliquot and add it to 200 µL 5 mg/mL 1-pyrenyldiazomethane (Molecular Probes, Eugene OR) in ethyl acetate, heat at 40° for 1 h, cool to room temperature, add to a Sep-Pak silica SPE cartridge, wash with 5 mL hexane, elute with 10 mL MeOH. Evaporate the eluate to dryness, reconstitute with 1 mL MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 µm LiChrosorb Si60
Mobile phase: MeCN:water 43:57 (or 57:43 (?))
Flow rate: 1
Injection volume: 5-10
Detector: F ex 340 em 395, UV 240

CHROMATOGRAM

Retention time: 23
Limit of detection: 100 fmole

KEY WORDS

derivatization; tablets; SPE; serum

REFERENCE

Yoshida,T.; Uetake,A.; Nakai,C.; Nimura,N.; Kinoshita,T. Liquid chromatographic determination of biotin using 1-pyrenyldiazomethane as a pre-column fluorescent labelling reagent, *J.Chromatogr.*, **1988**, *456*, 421–426.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 8.89

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: feed, formulations
Sample preparation: Formulations. Dilute 50 μL liquid vitamin to 2 mL with mobile phase, filter, inject a 20 μL aliquot. Feed. Extract 100 mg horse feed with 10 mL 1 M NaOH. Remove a 6 mL aliquot and adjust the pH to 6-7 with 1 M HCl, dilute to 100 mL with 100 mM pH 6.0 phosphate buffer, filter, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C18
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: A was 100 mM pH 6.0 phosphate buffer. B was MeOH:200 mM pH 6.0 phosphate buffer 50:50. A:B was 54:46.
Flow rate: 0.4
Injection volume: 20
Detector: F ex 490 em 520 (photon-counting) following post-column reaction. Column effluent mixed with a 2 μg/mL solution of avidin-FITC (Sigma) in 100 mM pH 7.0 phosphate buffer pumped at 1 mL/min. The mixture flowed through a 10 m long by 0.5 mm i.d. length of knitted open tubular PTFE tubing to the detector.

CHROMATOGRAM

Retention time: 15
Limit of detection: 4.45 ng/mL

OTHER SUBSTANCES

Extracted: biocytin
Noninterfering: DMF, acetone, methyl ethyl ketone, vitamin A, ascorbic acid, vitamin D, vitamin E, pyridoxine, vitamin B12, thiamine, riboflavin, niacin, pantothenic acid

KEY WORDS

liquid vitamin; horse feed; complexation; post-column reaction

REFERENCE

Przyjazny,A.; Hentz,N.G.; Bachas,L.G. Sensitive and selective liquid chromatographic postcolumn reaction detection system for biotin and biocytin using a homogeneous fluorophore-linked assay, *J.Chromatogr.A*, **1993**, *654*, 79–86.

SAMPLE

Matrix: formula

Sample preparation: 20 mL Infant formula + 150 μL concentrated HCl, filter (Whatman No. 1 paper), rinse precipitate with 1 mL water, adjust the pH of the filtrate to 7.0 with 6 M NaOH, extract four times with 8 mL n-hexane, dilute the aqueous layer to 25 mL with water, inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C18

Column: 250 × 4.6 5 μm Microsorb C18

Mobile phase: MeOH:100 mM pH 7.0 phosphate buffer 20:80

Flow rate: 0.4

Injection volume: 20

Detector: F ex 495 em 518 following post-column derivatization. The effluent from the column mixed with reagent pumped at 0.1 mL/min and flowed through a 10 m × 0.5 mm i.d. PTFE tube in a knitted open-tubular reactor. The reagent was 2 μg/mL streptavidin-FITC (streptavidin labeled with fluorescein isothiocyanate 1:3.6, Vector Laboratories) in 100 mM pH 9.5 phosphate buffer, prepared fresh daily.)

CHROMATOGRAM

Retention time: 14

Limit of detection: 97 pg

OTHER SUBSTANCES

Extracted: biocytin

Noninterfering: niacinamide

KEY WORDS

post-column reaction; complexation

REFERENCE

Hentz,N.G.; Bachas,L.G. Class-selective detection system for liquid chromatography based on the streptavidin-biotin interaction, *Anal.Chem.*, **1995**, *67*, 1014–1018.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets or capsules, weigh out amount corresponding to 200 μg biotin, add 30 mL water, sonicate for10 min, make up to 50 mL with water, centrifuge at 2000 g for 10 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Tomsorb C18 (Tomsic, Tokyo, Japan)

Mobile phase: MeCN:buffer 5:95 (Buffer was 20 mM sodium dodecyl sulfate adjusted to pH 3.5 with perchloric acid.)

Column temperature: 40

Flow rate: 0.5

Injection volume: 20

Detector: F ex 342 em 542 following post-column reaction. The column effluent mixed with 0.03% sodium hypochlorite in 200 mM pH 12.5 borate buffer pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm I.D. stainless steel tube at 50°. The effluent from this tube mixed with the reagent pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm I.D. stainless steel tube at 50° to the detector. (Prepare the reagent by dissolving 800 mg o-phthalaldehyde in 20 mL EtOH, adding 1.5 mL 3-mercaptopropionic acid and making up to 500 mL with 200 mM pH 10.5 borate buffer.)

CHROMATOGRAM

Retention time: 27

Limit of detection: 10 ng
Limit of quantitation: 20 ng

KEY WORDS
post-column reaction; tablets; capsules; granules

REFERENCE
Nojiri,S.; Kamata,K.; Nishijima,N. Fluorescence detection of biotin using post-column derivatization with OPA in high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1357–1362.

SAMPLE
Matrix: formulations
Sample preparation: Grind 20 tablets, weight out amount equivalent to 100 μg biotin, add 60 mL 1.5% phosphoric acid, sonicate for 20 min in a water bath at 50°, shake mechanically for 20 min, add 15 mL MeCN, dilute to 100 mL with 1.5% phosphoric acid, mix. Filter through a 0.45 μm nylon filter membrane, inject a 150 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm YMC Octylsilane C8 (YMC, Wilmington, NC)
Mobile phase: MeCN:water adjusted to pH 2.2 with phosphoric acid 5:95
Column temperature: 50
Flow rate: 2
Injection volume: 150
Detector: UV 200

CHROMATOGRAM
Retention time: 20.5
Limit of quantitation: 500 ng/mL

KEY WORDS
tablets

REFERENCE
Ekpe,A.E.; Hazen,C. Liquid chromatographic determination of biotin in multivitamin-multimineral tablets, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1311–1315.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve crushed tablet with sonication in 25 mL pH 3.5 potassium phosphate, centrifuge at 10000 rpm for 5 min, filter (0.45 μm) a 2 mL aliquot. Add filtrate to a C18 Sep-Pak SPE cartridge, wash with 2 mL water, wash with 3 mL phosphate buffer, wash with 2 mL MeCN:phosphate buffer 5:95, elute with MeCN:phosphate buffer 15:85, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Vydac HS C18
Mobile phase: MeCN:10 mM pH 3.5 KH_2PO_4 10:90
Detector: UV 230

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 75 μg/mL

KEY WORDS
tablets; SPE

REFERENCE
Hudson,T.S.; Subramanian,S.; Allen,R.J. Determination of pantothenic acid, biotin, and vitamin B12 in nutritional products, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 994–998.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: caffeine, citric acid, folic acid, niacin, niacinamide, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, 1993, **1993**,

SAMPLE
Matrix: solutions
Sample preparation: Evaporate biological samples, add 10 μmoles dibenzo-18-crown-6, add 100 μmoles 2,4'-dibromoacetophenone (p-bromophenacyl bromide), add 25 mg anhydrous potassium carbonate, add 1.6 mL MeCN, reflux for 1 h. For bulk quantities take 55 mg biotin + 5 mL EtOH + 3 mL water, neutralize (phenolphthalein) with 20 mM KOH in MeOH, remove the solvent. Suspend in 5 mL MeCN, add 60 mg potassium carbonate, add 24 mg dibenzo-18-crown-6, add 166 mg 2,4'-dibromoacetophenone, reflux for 1 h.

HPLC VARIABLES
Column: 330 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 60:40
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 17.4
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: dethiobiotin

KEY WORDS
derivatization

REFERENCE
Desbene,P.-L.; Coustal,S.; Frappier,F. Separation of biotin and its analogs by high-performance liquid chromatography: convenient labeling for ultraviolet or fluorimetric detection, *Anal.Biochem.*, **1983**, *128*, 359–362.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, pantothenic acid, riboflavin, niacinamide

REFERENCE
MetaChem Catalog, **1995**, p. 21.

SAMPLE
Matrix: tissue
Sample preparation: Condition two Sep-Pak C18 SPE cartridges with 10 mL MeOH and 10 mL water. 2-3 g Gut tissue or liver + 5 mL 5% trichloroacetic acid + 5 nmole dethiobiotin, homogenize, centrifuge at 10000 g for 15 min, re-extract pellet with 5 mL 5% trichloroacetic acid twice. Combine the supernatants and add them to a SPE cartridge, wash with 10 mL MeCN:water 2:98, elute with 10 mL MeCN:water 15:85, add the eluate to a 70 × 8 column of 200-400 mesh Dowex 1x8 formate, wash with 10 mL water, wash with 10 mL 100 mM potassium formate, elute with 30 mL 100 mM potassium formate. Add the eluate to a SPE cartridge, wash with 10 mL water, elute with 10 mL methyl formate. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL 2.5 mM panacyl bromide (p-(9-anthroyl)phenacyl bromide) and 0.5 mM dibenzo-18-crown-6 in acetone, add 20-30 mg potassium carbonate, heat at 57° for 3 h, inject an aliquot. (Panacyl bromide is available from Molecular Probes, Eugene OR.)

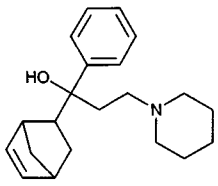
HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil
Mobile phase: Dichloromethane:MeOH 95:5
Flow rate: 1.4
Injection volume: 100
Detector: F ex 380 em 470

CHROMATOGRAM
Retention time: 6.46
Internal standard: dethiobiotin (5.56)

KEY WORDS
SPE; gut; rat; liver; normal phase

REFERENCE
Stein,J.; Hahn,A.; Lembcke,B.; Rehner,G. High-performance liquid chromatographic determination of biotin in biological materials after crown ether-catalyzed fluorescence derivatization with panacyl bromide, *Anal.Biochem.*, **1992**, *200*, 89–94.

Biperiden



Molecular formula: $C_{21}H_{30}ClNO$
Molecular weight: 311.47
CAS Registry No.: 514-65-8, 1235-82-1 (HCl), 7085-45-2 (lactate)
Merck Index: 1274
Lednicer No.: 1 47

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 10.0 sodium carbonate buffer + 5 mL diethyl ether, shake vigorously for 5 min, centrifuge at 2000 rpm for 5 min. Remove the organic phase and add it to 2 mL 1 M HCl, shake for 2 min, centrifuge at 2000 rpm for 5 min. Remove the aqueous phase and add it to 1 mL 3 M NaOH, add 5 mL diethyl ether, shake for 2 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. (Extraction procedure from Chem. Pharm. Bull. 1985, 33, 4581.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Detector: UV 199

CHROMATOGRAM

Retention time: 5.6

OTHER SUBSTANCES

Simultaneous: hyoscyamine, orphenadrine, benztropine
Noninterfering: amantadine, carbidopa, levodopa
Interfering: bromocriptine

KEY WORDS

plasma

REFERENCE

Selinger,K.; Lebel,G.; Hill,H.M.; Discenza,C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma, *J.Chromatogr.*, **1989**, *491*, 248–252.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.847

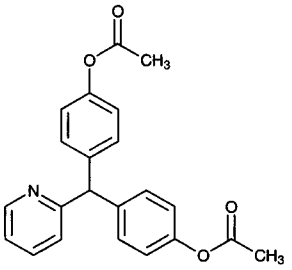
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bisacodyl



Molecular formula: $C_{22}H_{19}NO_4$
Molecular weight: 361.40
CAS Registry No.: 603-50-9, 1336-29-4 (complex with tannic acid)
Merck Index: 1282

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Grind tablets to powder, weigh out an amount equivalent to 5 mg bisacodyl, add 10-15 mL isopropanol, sonicate for 15 min, cool to room temperature, dilute to 25 mL with isopropanol, mix, centrifuge for 5 min. Remove a 5 mL aliquot and evaporate it to dryness under a stream of air, reconstitute the residue in 10 mL mobile phase, inject a 20 μL aliquot. Suppositories. Weigh out suppositories equivalent to about 50 mg bisacodyl, dissolve in warm (60°) isopropanol, cool to 15°, make up to 250 mL with isopropanol, mix, centrifuge an aliquot at 2000 rpm for 5 min. Remove 5 mL supernatant and evaporate it to dryness under a stream of air, dissolve in mobile phase, make up to 10 mL with mobile phase, centrifuge at 2000 rpm for 5 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:10 mM citric acid 25:25:50
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Limit of quantitation: 2500 ng/mL

KEY WORDS

tablets; suppositories

REFERENCE

Valenti,L.P.; Lau-Cam,C.A. Reverse phase liquid chromatographic determination of bisacodyl in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 529–532.

SAMPLE

Matrix: formulations
Sample preparation: Suppositories. Heat a suppository in 150 mL EtOH at 45° until it dissolves, cool to room temperature, make up to 200 mL with EtOH. Remove a 10 mL aliquot and add it to 20 mL mobile phase, filter (GF/C glass microfiber), inject a 20 μL aliquot. Tablets. Shake 10 tablets in 10 mL buffer until tablets are soft, break up with a glass rod, add 170 mL EtOH, sonicate for 25 min with occasional shaking, cool to room temperature, make up to 200 mL with EtOH. Remove a 10 mL aliquot and add it to 20 mL mobile phase, filter (GF/C glass microfiber), inject a 20 μL aliquot. Micro-enema. Sonicate micro-enema with 40 mL EtOH for 15 min, cool to room temperature, make up to 50 mL with EtOH. Remove a 10 mL aliquot and add it to 20 mL mobile phase, filter (GF/C glass microfiber), inject a 20 μL aliquot. (Buffer was 2.88 g Na_2HPO_4 and 1.145 g KH_2PO_4 in 100 mL water.)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 60 RP-select B

Mobile phase: MeCN:50 mM KH_2PO_4 55:45
Flow rate: 1
Injection volume: 20
Detector: UV 214

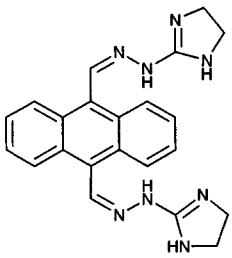
CHROMATOGRAM
Retention time: 7.1

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
suppositories; tablets; micro-enema

REFERENCE
Bradshaw,K.M.; Burnett,J.; Sidhu,A.S. High-performance liquid chromatographic determination of bisacodyl in pharmaceutical dosage forms marketed in Australia, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1355–1362.

Bisantrene



Molecular formula: $C_{22}H_{22}N_8$
Molecular weight: 398.47
CAS Registry No.: 78186-34-2, 71439-68-4 (di HCl)
Merck Index: 1284
Lednicer No.: 4 62

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Incubate 500 μL Bile or urine +50 μL 1 M pH 4.0 sodium acetate + 10 U/mL glucuronidase overnight. 2 mL Plasma or a lesser volume of urine or bile diluted to 1 mL with water + an equal volume of 1 M pH 10 sodium phosphate buffer + 1 μg IS + 8 mL ethyl acetate, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeOH with gentle warming, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 mm long 5 μm LiChrosorb RP-2 C2
Mobile phase: Gradient. MeOH:500 mM pH 5.3 sodium perchlorate from 5:95 to 100:0 over 15 min. (Purify sodium perchlorate solution by passing through a bed of silica gel and activated charcoal before use.)
Flow rate: 2
Injection volume: 100
Detector: UV 430 for bisantrene, UV 500 for IS

CHROMATOGRAM

Retention time: 7
Internal standard: 1-[2-(2-hydroxyethyl-1-amino)ethylamino]-4-hydroxy-9,10-anthracenedione
Limit of detection: 50 ng/mL (human plasma), 25 ng/mL (rabbit plasma)

KEY WORDS

plasma; rabbit; pharmacokinetics; human

REFERENCE

Powis,G. Reversed-phase high-performance liquid chromatographic assay for the antineoplastic agent 9,10-anthracenedicarboxaldehyde bis(4,5-dihydro-1H-imidazol-2-yl hydrazone) dihydrochloride, *J.Chromatogr.*, **1981**, *226*, 514–520.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 5% perchloric acid, mix, add 100 μL 25% ammonium hydroxide, add 4 mL ethyl acetate, vortex for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL 500 mM HCl, inject an aliquot.

HPLC VARIABLES

Column: Reversed-phase C18 (Waters or Bio-Rad)
Mobile phase: MeOH:200 mM pH 4.0 ammonium acetate 40:60
Flow rate: 2
Detector: UV 260

CHROMATOGRAM

Retention time: 7

Limit of detection: 30 ng/mL

KEY WORDS
plasma

REFERENCE
Davis,T.P.; Peng,Y.-M.; Goodman,G.E.; Alberts,D.S. HPLC, MS, and pharmacokinetics of melphalan, bisantrene and 13-cis retinoic acid, *J.Chromatogr.Sci.*, **1982**, *20*, 511–516.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 10 mL MeOH and 5 mL water. Add 1-2 mL plasma to the SPE cartridge, wash with 5 mL water, elute with 300 μL 500 mM methanolic HCl, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS (Whatman)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:200 mM pH 4.0 ammonium acetate 27:73
Flow rate: 2
Detector: UV 436

CHROMATOGRAM
Retention time: 4
Limit of detection: 10 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Peng,Y.-M.; Ormberg,D.; Alberts,D.S.; Davis,T.P. Improved high-performance liquid chromatography of the new antineoplastic agents bisantrene and mitoxantrone, *J.Chromatogr.*, **1982**, *233*, 235–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL chloroform:MeOH:6 M HCl 83:16:1, agitate, centrifuge at 2000 g for 5 min, discard the organic phase, extract the aqueous phase with 3 mL chloroform and 100 μL 28% ammonium hydroxide, agitate, centrifuge. Remove 2.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, agitate, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:ammonium formate 30:60:5 (v/v/v)
Flow rate: 2
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 2.5
Internal standard: CL 238,985 (American Cyanamid analog of bisantrene) (4)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Weiss,G.R.; Hersh,M.; Kuhn,J.G.; Ludden,T.M.; von Hoff,D.D.; Kisner,D.L.; Pirtle,T.E.,III A phase I and pharmacokinetic comparison of hepatic arterial and peripheral vein infusions of bisantrene for liver cancer, *Cancer Chemother.Pharmacol.*, **1985**, *15*, 144–148.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of 1 mL plasma to 11 with 50 μL 1 M NaOH, add 5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, centrifuge at 15600 g for 1 min, inject a 150 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 37-50 μm C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:80 mM pH 3.0 sodium formate 28:72
Flow rate: 1
Injection volume: 150
Detector: E, BAS LC-4B detector, TL-5 glassy carbon electrode at +0.75 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8
Internal standard: bisantrene

OTHER SUBSTANCES
Extracted: mitoxantrone

KEY WORDS
plasma; pharmacokinetics; bisantrene is IS

REFERENCE
Choi,K.E.; Sinkule,J.A.; Han,D.S.; McGrath,S.C.; Daly,K.M.; Larson,R.A. High-performance liquid chromatographic assay for mitoxantrone in plasma using electrochemical detection, *J.Chromatogr.*, **1987**, *420*, 81–88.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 4 mL MeOH, 4 mL MeOH:water 50:50, and 10 mL 50 mM 50 mM sodium phosphate. Add 3 mL plasma or urine to the SPE cartridge, wash with 4 mL 50 mM sodium phosphate, elute with 6 mL chloroform:MeOH 2:1. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL N,N-dimethylacetamide and 250 μL saline, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60 containing 20 mM ammonium acetate, pH 4.0
Flow rate: 1.5
Detector: F ex 260 em 550

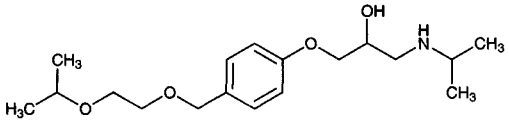
CHROMATOGRAM
Retention time: 8.5
Limit of detection: 2 ng/mL

KEY WORDS
plasma; SPE

REFERENCE

Lu,K.; Savaraj,N.; Huang,M.T.; Moore,D.; Loo,T.L. High performance liquid chromatography (HPLC) of the new antineoplastic 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1H-imidazole-2-yl)hydrazone] dihydrochloride (CL 216,942; bisantrene), *J.Liq.Chromatogr.*, **1982**, *5*, 1323–1328.

Bisoprolol



Molecular formula: $C_{18}H_{31}NO_4$
Molecular weight: 325.45
CAS Registry No.: 66722-44-9, 104344-23-2 (fumarate)
Merck Index: 1336
Lednicer No.: 4 28

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL pH 9.2 bicine (N,N-bis(2-hydroxyethyl)glycine) + 4 mL ethyl acetate, shake vigorously for 5 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, inject an aliquot of 100 μL or less.

HPLC VARIABLES

Guard column: 10 mm long LiChrosorb CN
Column: 250 × 4 10 μm LiChrosorb CN
Mobile phase: MeOH:isopropanol:1.16 M perchloric acid 75:25:0.5
Flow rate: 2.5
Injection volume: ≤100
Detector: F ex 235 em 310

CHROMATOGRAM

Retention time: 3.6
Internal standard: bisoprolol

OTHER SUBSTANCES

Extracted: sotalol
Simultaneous: verapamil
Noninterfering: acebutolol, amiodarone, disopyramide, propafenone, hydroquinidine, quinidine

KEY WORDS

plasma; bisoprolol is IS; pharmacokinetics

REFERENCE

Poirier,J.-M.; Lebot,M.; Cheymol,G. Rapid and sensitive column liquid chromatographic determination of sotalol in plasma, *J.Chromatogr.*, **1989**, *493*, 409–413.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30

Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 5.84
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a Bond Elut SI SPE cartridge with 3 mL chloroform. 1 mL Plasma or 500 μL urine + 200 (plasma) or 500 (urine) μL water + 200 (plasma) or 300 (urine) μL 100 mM NaOH + 7 mL chloroform, shake for 10 min, centrifuge at 1800 g for 5 min. Remove the organic layer and add it to the SPE cartridge, wash with 1 mL MeOH, elute with 1 mL MeOH:triethylamine 95:5. Evaporate the eluate to dryness under

a stream of nitrogen at 60°, reconstitute the residue in 200 μL IS solution, inject a 100 μL aliquot. (Prepare IS solution by repeatedly injecting high concentrations of IS solution onto this HPLC system, collect the third peak, evaporate the collected eluates to dryness under a stream of nitrogen, reconstitute with mobile phase to an appropriate concentration.)

HPLC VARIABLES

Guard column: 50 × 4.6 10 μm Chiralcel OD (Daicel)
Column: 250 × 4.6 10 μm Chiralcel OD (Daicel)
Mobile phase: Hexane:isopropanol 100:9 containing 0.01% diethylamine
Column temperature: 25
Flow rate: 0.5
Injection volume: 100
Detector: F ex 228 em 298

CHROMATOGRAM

Retention time: 20 (R(+)), 30 (S(-))
Internal standard: 1-[p-(tetrahydro-3-furanyl)phenoxy]-3-isopropylamino-2-propanol (37)
Limit of detection: 2 ng/mL

KEY WORDS

plasma; chiral; SPE

REFERENCE

Suzuki,T.; Horikiri,Y.; Mizobe,M.; Noda,K. Sensitive determination of bisoprolol enantiomers in plasma and urine by high-performance liquid chromatography using fluorescence detection, and application to preliminary study in humans, *J.Chromatogr.*, **1993**, *619*, 267–273.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 12.283

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 50:50 (Buffer was 50 mM $(NH_4)_2HPO_4$ adjusted to pH 7.0 with 5 M orthophosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Retention time: 7
Limit of detection: 5-20 ng

OTHER SUBSTANCES
Simultaneous: fumaric acid, impurities

REFERENCE
Agapova,N.N.; Vasileva,E. High-performance liquid chromatographic method for the determination of bisoprolol and potential impurities, *J.Chromatogr.A*, **1993**, *654*, 299–302.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 3.33 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.85

OTHER SUBSTANCES
Also analyzed: carvedilol, labetalol, metipranolol, oxprenolol, talinolol, toliprolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD

Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.69, 1.38 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

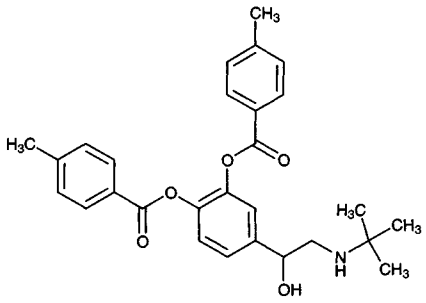
CHROMATOGRAM
Retention time: k' 4.43

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Bitolterol

Molecular formula: $C_{28}H_{31}NO_5$
Molecular weight: 461.56
CAS Registry No.: 30392-40-6, 30392-41-7 (mesylate)
Merck Index: 1344
Lednicer No.: 3 22



SAMPLE
Matrix: formulations
Sample preparation: Discharge aerosol into 10 mL MeOH in a 50 mL beaker until about 1.2 g sample is collected, rinse aerosol outlet with MeOH, determine weight of sample by difference in weight of can, dilute with MeCN:water:acetic acid 60:38:2 to about 20 μg/mL bitolterol, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeCN:water:acetic acid:sodium octanesulfonate 600:380:20:0.65 (v/v/v/w)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: colterol, degradation products

KEY WORDS
aerosols

REFERENCE
Wilson,T.D.; Fogarty,D.F. The effect of column age on system suitability parameters for an HPLC assay of bitolterol mesylate aerosols, *J.Chromatogr.Sci.*, **1988**, *26*, 60–66.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeCN:water:glacial acetic acid:sodium octanesulfonate 600:380:20:0.65
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7 (mesylate)

OTHER SUBSTANCES
Simultaneous: impurities, colterol

KEY WORDS
stability-indicating

REFERENCE

Wilson,T.D. High-performance liquid chromatographic determination of Tornalate in solution dosage forms; a specificity study, *J.Chromatogr.*, **1987**, *391*, 409–418.

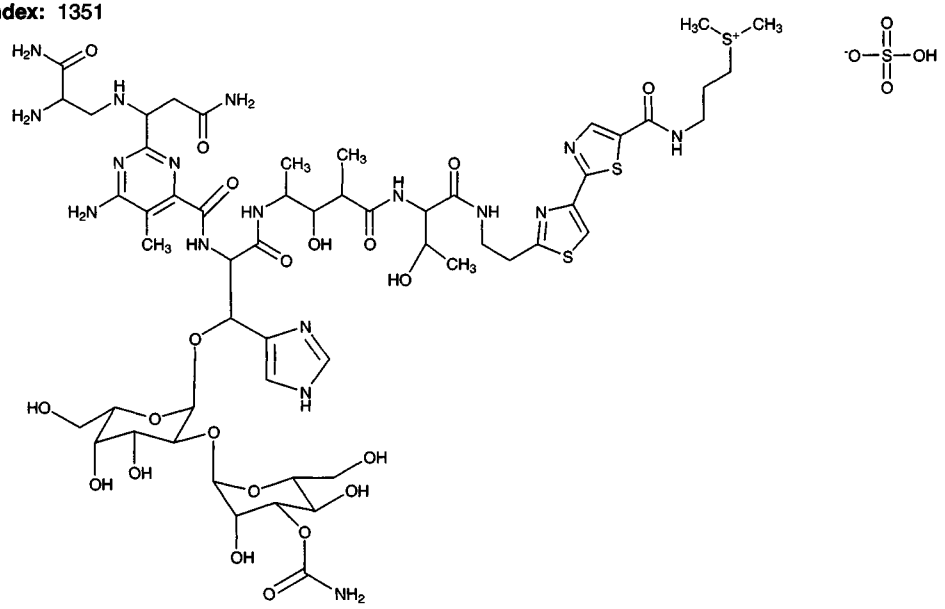
Bleomycin sulfate

Molecular formula: $C_{55}H_{84}N_{17}O_{21}S_3$ (bleomycin A_2)

Molecular weight: 1415.59

CAS Registry No.: 9041-93-4, 11056-06-7 (free base), 58995-26-9 (bleomycin A_1), 11116-31-7 (bleomycin A_2), 11116-32-8 (bleomycin A_5), 37293-17-7 (bleomycin A_6), 41138-54-9 (bleomycin B_1), 9060-10-0 (bleomycin B_2), 9060-11-1 (bleomycin B_4), 73666-80-5 (bleomycin B_6)

Merck Index: 1351



SAMPLE

Matrix: blood, hepatocytes

Sample preparation: 300 μL Plasma or hepatocyte suspension + 0.9 μg peplomycin + 75 μL trichloroacetic acid:water 20:80 containing 1 mM copper sulfate, vortex for 1 min, centrifuge at 850 g for 10 min, rewash the pellet with 75 μL trichloroacetic acid:water 20:80 containing 1 mM copper sulfate, vortex for 1 min, centrifuge at 850 g for 10 min. Combine the supernatants, make up to 300 μL with water, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 37 × 3.9 Corasil C18

Column: 250 × 10 7 μm Lichrosorb RP-18

Mobile phase: Gradient. A was 5 mM sodium pentanesulfonate in 0.5% acetic acid, adjusted to pH 4.3 with 28% ammonia. B was MeOH:MeCN 25:75 containing 5 mM sodium pentanesulfonate and 0.5% acetic acid. A:B from 87:13 to 67:33 over 20 min

Column temperature: 35

Flow rate: 1.8

Injection volume: 100

Detector: F ex 297 em 355

CHROMATOGRAM

Retention time: 7 (deamidobleomycin A2), 8 (bleomycin A2), 9 (deamidobleomycin B2), 10 (bleomycin B2)

Internal standard: peplomycin (14.5)

Limit of detection: 70 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Mahdadi,R.; Kenani,A.; Pommery,N.; Pommery,J.; Hénichart,J.P.; Lhermitte,M. High-performance liquid chromatography assay of bleomycin in human plasma and rat hepatocytes in culture, *Cancer Chemother.Pharmacol.*, **1991**, *28*, 22–26.

SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 10000 ng/mL

REFERENCE
Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4.6 μBondapak C18
Mobile phase: Gradient. All solvents contained 5 mM ammonium formate. MeOH:water 15:85 for 2 h, to 30:70 over 1 h, to 95:5 over 1.5 h.
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 80 (bleomycin A2), 150 (bleomycin B2), 200 (bleomycin A1), 240 demethylbleomycin A2)

REFERENCE
Rzeszotarski,W.J.; Eckelman,W.C.; Reba,R.C. Reversed-phase high-performance liquid chromatography of bleomycin, *J.Chromatogr.*, **1976**, *124*, 88–91.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4.6 μBondapak C18
Mobile phase: MeOH:water 50:50 containing 5 mM heptanesulfonic acid, adjust pH to 8.3 with concentrated ammonium hydroxide
Flow rate: 1.5
Detector: UV 290

CHROMATOGRAM
Retention time: 6 (bleomycin B2), 12 (bleomycin A2)
Limit of detection: 10 pmole

REFERENCE
Sakai,T.T. Paired-ion high-performance liquid chromatography of bleomycins, *J.Chromatogr.*, **1978**, *161*, 389–392.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in water, add a slight molar excess of copper sulfate, inject a 10-20 μL aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 30-38 μm Corasil C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 5 mM sodium pentanesulfonate in 0.5% glacial acetic acid in water, adjusted to pH 4.3 with concentrated ammonium hydroxide. B was MeOH containing 5 mM sodium pentanesulfonate and 0.5% glacial acetic acid. A:B from 72:28 to 52:48 over 45 min.
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 280

CHROMATOGRAM
Retention time: 5 (bleomycinic acid), 10 (bleomycin B'1), 11.5 (bleomycin A2), 12.5 (bleomycin A5), 17.5 (bleomycin B2), 22.5 (bleomycin B4), 25.5 (bleomycin B6), 29 (bleomycin CHP), 32 (bleomycin PEPP), 35 (demethylbleomycin A2)
Limit of detection: 50 pmole

KEY WORDS
chelates

REFERENCE
Klett,R.P.; Chovan,J.P.; Danse,I.H.R. Reversed-phase paired-ion high-performance liquid chromatographic method for the separation and quantification of multiple bleomycin congeners, *J.Chromatogr.*, **1984**, *310*, 361–371.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in water, add a slight molar excess of copper sulfate, inject a 10-20 μL aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 30-38 μm Corasil C18
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was 5 mM sodium heptanesulfonate in 0.5% glacial acetic acid in water, adjusted to pH 4.3 with concentrated ammonium hydroxide. B was MeOH containing 5 mM sodium heptanesulfonate and 0.5% glacial acetic acid. A:B from 40:60 to 50:50 over 30 min.
Flow rate: 0.7
Injection volume: 10-20
Detector: UV 280

CHROMATOGRAM
Retention time: 5 (epibleomycin A2), 8 (epibleomycin B2), 11 (desamidobleomycin A2), 12 (bleomycin A2), 14 (isobleomycin A2), 18 (desamidobleomycin B2), 20 (bleomycin B2), 21 (isobleomycin B2), 22 (epidemethylbleomycin A2), 24 (desamidodemethylbleomycin A2), 25.5 (demethylbleomycin A2), 28 (isodemethylbleomycin A2)

KEY WORDS
chelates

REFERENCE
Klett,R.P.; Chovan,J.P. Modification of a new high-performance liquid chromatographic method for bleomycin to separate epi-, iso-, desamido-, and unmodified analogs, *J.Chromatogr.*, **1985**, *337*, 182–186.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 50 × 5 10 μm Mono S HR 5/5 cation-exchange (Pharmacia LKB)
Mobile phase: Gradient. A was 50 mM pH 6.5 ammonium formate. B was 1 M pH 6.5 ammonium formate. A:B from 98:2 to 95:5 over 30 min, to 75:25 over 20 min, to 0:100 over 10 min.
Column temperature: 4
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 12 (bleomycin A2), 41 (bleomycin B2)

REFERENCE
Mistry,J.S.; Sebti,S.M.; Lazo,J.S. Separation of bleomycins and their deamido metabolites by high-performance cation-exchange chromatography, *J.Chromatogr.*, **1990**, *514*, 86–90.

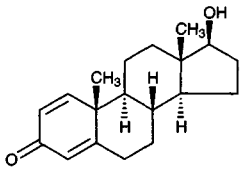
SAMPLE
Matrix: solutions
Sample preparation: Make up a 1 mg/mL (1 U/mL) aqueous solution, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher RP-Select B
Column: 125 × 4 5 μm LiChrospher RP-Select B
Mobile phase: Gradient. A was 10 mM sodium perchlorate in 0.1% aqueous phosphoric acid. B was MeCN. A:B from 95:5 to 75:25 over 13 min, to 0:100 over 1 min, maintain at 0:100 for 1 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5 (bleomycin A2), 7.8 (bleomycin B2), 12.3 (demethylbleomycin A2)
Limit of detection: 10 μg/mL

REFERENCE
Fiedler,H.-P.; Wachter,J. High-performance liquid chromatographic determination of bleomycins, *J.Chromatogr.*, **1991**, *536*, 343–347.

Boldenone



Molecular formula: $C_{19}H_{26}O_2$
Molecular weight: 286.41
CAS Registry No.: 846-48-0, 13103-34-9 (undecylenate)
Merck Index: 1354
Lednicer No.: 2 153

SAMPLE

Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone
Interfering: fluoxymesterone, norethindrone, oxandrolone (UV 210), ethisterone

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 4.5 (boldenone undecylenate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: testosterone undecenoate, nandrolone decanoate, methandriol dipropionate, testosterone decanoate, nandrolone laurate, testosterone undecanoate, testosterone, methenolone acetate, testosterone propionate, nandrolone phenylpropionate, testosterone phenylpropionate, testosterone isocaproate, trenbolone hexahydrobenzylcarbonate
Interfering: testosterone enanthate, methenolone enanthenate, testosterone cypionate

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 6.8 (boldenone), 13.4 (boldenone acetate), 25.1 (boldenone undecylenate)

OTHER SUBSTANCES
Simultaneous: clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.; Sperling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 75:25
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5 (boldenone), 5.5 (boldenone acetate), 16 (boldenone benzoate)

OTHER SUBSTANCES
Also analyzed: nandrolone, nandrolone propionate, nandrolone phenylpropionate

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: methyltestosterone, nandrolone, methandrostenolone, testosterone, danazol, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 μg/mL solution in MeOH.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM ammonium acetate buffer 45:55
Flow rate: 0.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.091

OTHER SUBSTANCES
Simultaneous: epimethandienone, epitestosterone, fluoxymesterone, 6β-hydroxymethandienone, methandienone, norethindrone, oxymetholone (UV 280), trenbolone

REFERENCE
Barrón,D.; Pascual,J.A.; Segura,J.; Barbosa,J. Prediction of LC retention of steroids using solvatochromic parameters, *Chromatographia*, **1995**, *41*, 573–580.

SAMPLE
Matrix: urine
Sample preparation: Condition a 200 mg 40 μm C18 SPE cartridge (J.T.Baker model 7020-2) with 2 mL MeOH and 1 mL 25 mM pH 5.5 ammonium acetate (A). Condition a 200 mg 40 μm C18 SPE cartridge (J.T.Baker model 7020-2) with 2 mL MeOH and 1 mL water (B). 2 mL Urine + 2 mL MeOH:650 mM pH 5.4 ammonium acetate 20:80 + 75 μL 25.3 μM 19-nortestosterone sodium sulfate in water, sonicate for 15 min, centrifuge at 1000 g for 5 min, add to SPE cartridge (A), wash with 1 mL 25 mM pH 5.5 ammonium acetate, wash with 2 mL MeOH:25 mM pH 5.5 ammonium acetate 40:60, wash with 3 mL water, elute with 2 mL MeOH:water 35:65. Add 2 mL MeOH to the eluate and evaporate it under a stream of nitrogen at 60°, dissolve the residue in 100 μL MeOH, add 5 mL ethyl acetate saturated with 2.2 M sulfuric acid (one tenth volume), heat at 50° for 50 min, cool, wash twice with 2 mL 940 mM pH 10.3 sodium carbonate (pH adjusted with sodium bicarbonate), wash with 3 mL water. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, transfer the residue with three 200 μL portions of MeOH:water 50:50 to SPE cartridge (B), wash with 1 mL water, wash with 2 mL MeOH:water 55:45, elute with 2 mL MeOH:water 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Boldenone sulfate, isolated using SPE cartridge (A), is solvolysed to boldenone which is purified using SPE cartridge (B).)

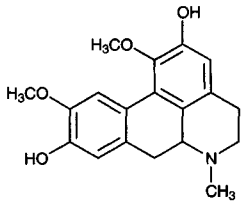
HPLC VARIABLES
Column: 83 × 4.6 3 μm Pecosphere-3C C18
Mobile phase: MeCN:MeOH:25 mM pH 5.5 ammonium acetate 7:50:43
Flow rate: 1.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.2
Internal standard: 19-nortestosterone sodium sulfate (7.2) (chromatographed as 19-nortestosterone)
Limit of detection: 64 ng/mL
Limit of quantitation: 212 ng/mL

KEY WORDS
horse; pharmacokinetics; SPE

REFERENCE
Weidolf,L.O.G.; Chichila,T.M.P.; Henion,J.D. Screening, confirmation and quantification of boldenone sulfate in equine urine after administration of boldenone undecylenate (Equipoise™), *J.Chromatogr.*, **1988**, *433*, 9–21.

Boldine



Molecular formula: $C_{19}H_{21}NO_4$
Molecular weight: 327.38
CAS Registry No.: 476-70-0
Merck Index: 1355

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM

Retention time: 8.068

KEY WORDS

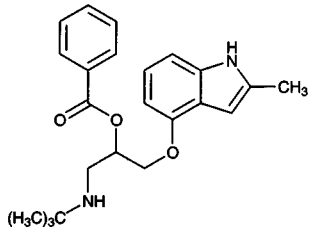
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bopindolol

Molecular formula: $C_{23}H_{28}N_2O_3$
Molecular weight: 380.49
CAS Registry No.: 62658-63-3
Merck Index: 1362



SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 80 ng/mL mepindolol in 100 mM acetic acid + 100 µL 1 M NaOH, vortex for 10 s, centrifuge at 1400 g for 4 min, add the supernatant to a 1 mL Extrelut R SPE cartridge, wait for 15 min, elute with 6 mL heptane:isoamyl alcohol 95:5. Add 200 µL 100 mM acetic acid to the eluate, shake for 15 min, centrifuge at 1400 g for 5 min, remove the aqueous phase and keep it at 4°, inject a 100 µL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 83 × 4.6 3 µm Nucleosil ODS
Mobile phase: MeOH:30 mM pH 2.2 KH_2PO_4 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: E, ESA Model 5100 A, 5020 guard cell at +1 V (between pump and injector), 5011 dual analytical cell +160 and +450 mV, +450 mV cell monitored

CHROMATOGRAM

Retention time: 4.7 (for bopindolol hydrolysis product, 4-(2-hydroxy-3-tert-butylaminopropyl)-2-methylindole)
Internal standard: mepindolol (3.3) (structure shown is 3-methylpindolol, not 2-methylpindolol=mepindolol)
Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES

Noninterfering: digoxin, phenprocoumon, chlorthalidone

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Humbert,H.; Denouel,J.; Keller,H.P. Column liquid chromatographic determination of hydrolysed bopindolol, in the picogram per millilitre range in plasma, using cartridge extraction and dual electrochemical detection, *J.Chromatogr.*, **1987**, *422*, 205–215.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 70 µL 1 M NaOH + 25 µL 83.2 ng/mL IS in 100 mM acetic acid, vortex for 5 s, add 5 mL heptane:isoamyl alcohol 95:5, shake for 15 min on a horizontal shaker, centrifuge at 1400 g for 10 min. Remove 4.2 mL of the organic phase and add it to 150 µL 100 mM acetic acid, shake for 15 min on a horizontal shaker, centrifuge at 1400 g for 10 min, inject a 50 µL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 µm Pierce RP-18
Column: 150 × 4.6 5 µm Ultrasphere ODS C18

Mobile phase: MeOH:30 mM potassium phosphate, adjusted to pH 2.2 with phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, model 5010 analytical cells, detector 1 +150 mV, detector 2 +550 mV, monitor cell 2

CHROMATOGRAM
Retention time: 14 (as hydrolysis product)
Internal standard: 2-hydroxy-N-isopropyl-3-(3-methyl-1H-indol-4-yloxy)-1-propylamine (17-895, 3-methylpindolol) (9)
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Noninterfering: allopurinol, amphogel, aspirin, azathioprine, calcitonin, calcitriol, captopril, cimetidine, cloxacillin, cyclosporine, digoxin, diltiazem, ergoloid, furosemide, glyburide, loperamide, nifedipine, nitroglycerin, prazosin, prednisone, propranolol, sulfinpyrazone, tolbutamide

KEY WORDS
plasma

REFERENCE
Perkins,S.L.; Tattrie,B.; Johnson,P.M.; Rabin,E.Z. Analytical problems encountered during high-performance liquid chromatographic separation and coulometric detection of bopindolol metabolites in human plasma, *Ther.Drug Monit.*, **1988**, *10*, 480–485.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 5.35

OTHER SUBSTANCES
Also analyzed: alprenolol, betaxolol, propranolol, tertatolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80

Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.86

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

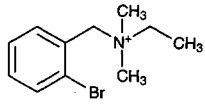
CHROMATOGRAM
Retention time: 12.5
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, amphetamine, atenolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

KEY WORDS
column-switching

REFERENCE
Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

Bretylium tosylate



Molecular formula: $C_{18}H_{24}BrNO_3S$
Molecular weight: 414.4
CAS Registry No.: 61-75-6, 59-41-6 (free base)
Merck Index: 1395
Lednicer No.: 1 55

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond-Elut CBA cation-exchange SPE cartridge with 3 mL MeOH, 3 mL water, 1 mL 50 mM pH 9.0 borate. 250 μL Plasma + 200 μL 1 μg/mL D-tubocurarine, add to SPE cartridge, wash with 3 mL water, wash with 1 mL 50 mM pH 3.0 NaH_2PO_4, wash with 1 mL water, wash with two 500 μL portions of MeOH, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL MeOH:MeCN:water 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid, inject a 70 μL aliquot. (Acidified MeOH was 833 μL HCl in 100 mL MeOH.); SPE

HPLC VARIABLES

Column: 100 × 4.9 5 μm octyl Spherisorb
Mobile phase: MeOH:MeCN:buffer 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid (Buffer was 10 mM sodium octanesulfonate and 1.5 mM dibutylamine.)
Flow rate: 2.5
Injection volume: 70
Detector: UV 272

CHROMATOGRAM

Retention time: 3
Internal standard: D-tubocurarine (5)
Limit of quantitation: 625 ng/mL

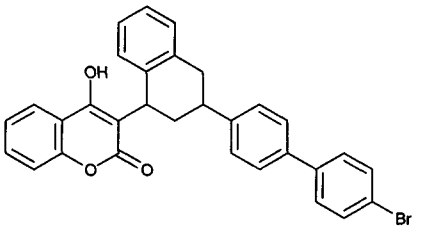
KEY WORDS

plasma; SPE; human; pig; pharmacokinetics

REFERENCE

Théorêt,Y.; Varin,F. Simple, rapid and selective method using high-performance liquid chromatography for the determination of bretylium in plasma, *J.Chromatogr.*, **1992**, *575*, 162–166.

Brodifacoum



Molecular formula: $C_{31}H_{23}BrO_3$
Molecular weight: 523.43
CAS Registry No.: 56073-10-0
Merck Index: 1400

SAMPLE

Matrix: blood, tissue

Sample preparation: Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 μg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH:chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 200 mm long μBondapak C18
Mobile phase: MeOH:0.8% acetic acid 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 17.8
Internal standard: N,N-diphenylbenzidine (9.3)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: bromadiolone, coumarin, coumatetralyl, warfarin

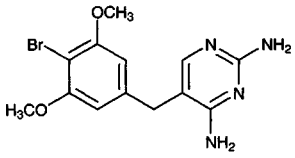
KEY WORDS

SPE; plasma; heart; lung; liver; kidney; spleen

REFERENCE

Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685–688.

Brodimoprim



Molecular formula: $C_{13}H_{15}BrN_4O_2$
Molecular weight: 339.19
CAS Registry No.: 56518-41-3
Merck Index: 1401

SAMPLE

Matrix: blood, urine
Sample preparation: Dilute urine 1:10 with water. 200 μL Plasma, blood, or diluted urine + 50 μL 5 μg/mL IS in MeOH + 500 μL 400 mM borate buffer + chloroform, vortex for a few s, centrifuge at 1700 g at 4° for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, let stand for at least 1 h at room temperature, inject a 20-50 μL aliquot. (Deconjugate 200 μL plasma or diluted urine with 100 μL 68 U/mL β-glucuronidase in 500 mM pH 5.0 sodium acetate buffer, heat at 37° for 4 h, add 100 μL 400 mM pH 10 borate buffer, add 50 μL 5 μg/mL IS in MeOH, extract as before.) (Use chloroform stabilized with EtOH.)

HPLC VARIABLES

Guard column: 10 × 3 30 μm Chrompack guard column
Column: 33 × 4.6 3 μm Pecosphere-3 × 3 CR C18
Mobile phase: MeCN:25 mM phosphate buffer 23:77 + 0.1% triethylamine, adjusted to pH 7.5 with 10 M KOH
Flow rate: 1
Injection volume: 20-50
Detector: F ex 290 em 340

CHROMATOGRAM

Retention time: 1.1
Internal standard: 2,4-diamino-5-(4'-(2-propenyl)-3',5'-dimethoxybenzyl)pyrimidine (Ro-11-3296) (4.2)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: flecainide
Noninterfering: trimethoprim, β-blockers

KEY WORDS

plasma; use UV detection with clonazepam IS and LOD 100 ng/mL

REFERENCE

Gaspari,F.; Taiocchi,L.; Pochobradsky,M.G. Determination of brodimoprim and its hydroxy metabolite in human plasma, blood and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *577*, 123–128.

SAMPLE

Matrix: blood, urine
Sample preparation: Extract plasma or urine with n-butyl acetate:MeOH 98:2, inject an aliquot of the organic layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: 12.5 mL MeOH + 1.5 mL 33% ammonia made up to 250 mL with 2-pentanone

Flow rate: 2.5
Injection volume: 100
Detector: F ex 290 em 340

CHROMATOGRAM
Retention time: 4.3
Internal standard: Ro 11-8958 (3.2)
Limit of quantitation: 40 ng/mL

KEY WORDS
plasma; normal phase; pharmacokinetics

REFERENCE
Weidekamm,E. Pharmacokinetics of brodimoprim, *J.Chemother.*, **1993**, *5*, 475–479.

SAMPLE
Matrix: cell suspensions
Sample preparation: Cool cell suspension in an ice bath, centrifuge at 800 g at 4° for 15 min, inject an aliquot of the supernatant.

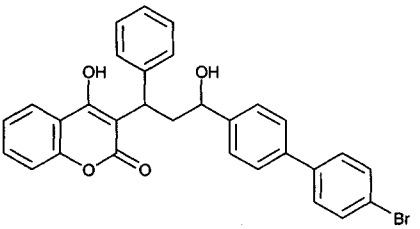
HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:MeOH:water 10:30:60 containing 10 mM K_2HPO_4
Flow rate: 2
Detector: UV 280

CHROMATOGRAM
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Also analyzed: trimethoprim

REFERENCE
Climax,J.; Lenehan,T.J.; Lambe,R.; Kenny,M.; Caffrey,E.; Darragh,A. Interaction of antimicrobial agents with human peripheral blood leucocytes: uptake and intracellular localization of certain sulphonamides and trimethoprims, *J.Antimicrob.Chemother.*, **1986**, *17*, 489–498.

Bromadiolone



Molecular formula: $C_{30}H_{23}BrO_4$
Molecular weight: 527.41
CAS Registry No.: 28772-56-7
Merck Index: 1403

SAMPLE

Matrix: blood, tissue

Sample preparation: Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 μg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH:chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 200 mm long μBondapak C18
Mobile phase: MeOH:0.8% acetic acid 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 7.1
Internal standard: N,N-diphenylbenzidine (9.3)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: brodifacoum, coumarin, coumatetralyl, warfarin

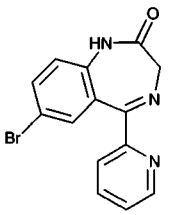
KEY WORDS

SPE; plasma; heart; lung; liver; kidney; spleen

REFERENCE

Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685–688.

Bromazepam



Molecular formula: $C_{14}H_{10}BrN_3O$
Molecular weight: 316.16
CAS Registry No.: 1812-30-2
Merck Index: 1406

SAMPLE

Matrix: blood

Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 6.6 (A), 25.5 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 234

CHROMATOGRAM

Retention time: 3.67
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 232.2

CHROMATOGRAM
Retention time: 14.732

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

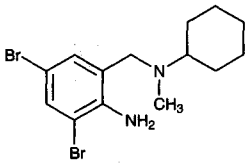
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Bromhexine



Molecular formula: $C_{14}H_{20}Br_2N_2$
Molecular weight: 376.13
CAS Registry No.: 3572-43-8, 611-75-6 (HCl)
Merck Index: 1412
Lednicer No.: 2 96

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.2

OTHER SUBSTANCES

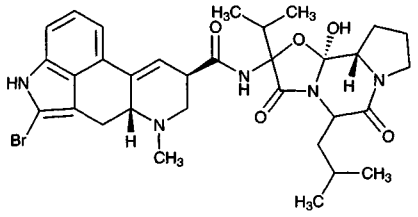
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-

thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Bromocriptine



Molecular formula: $C_{32}H_{40}BrN_5O_5$
Molecular weight: 654.60
CAS Registry No.: 25614-03-3, 22260-51-1 (mesylate)
Merck Index: 1437

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.652

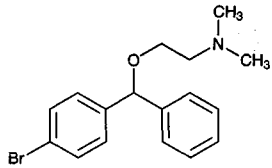
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bromodiphenhydramine



Molecular formula: $C_{17}H_{20}BrNO$
Molecular weight: 334.26
CAS Registry No.: 118-23-0, 1808-12-4 (HCl)
Merck Index: 1439
Lednicer No.: 1 42

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.4

OTHER SUBSTANCES

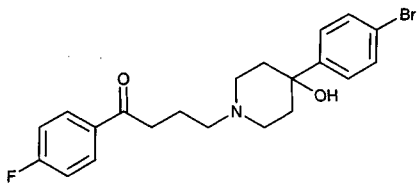
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Bromperidol



Molecular formula: $C_{21}H_{23}BrFNO_2$
Molecular weight: 420.32
CAS Registry No.: 10457-90-6
Merck Index: 1466
Lednicer No.: 2 331

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

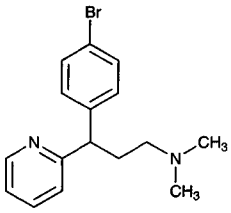
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Brompheniramine



Molecular formula: $C_{16}H_{19}BrN_2$
Molecular weight: 319.3
CAS Registry No.: 86-22-6, 132-21-8 (d-form), 980-71-2 (maleate), 2391-03-9 (d-form maleate)
Merck Index: 1467
Lednicer No.: 1 77

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 0.5 ng/mL (sic) antazoline hydrochloride in water + 5 mL freshly distilled ether, vortex for 1-2 s, add 200 μL 10% KOH, vortex for 15 s, centrifuge for 3-3 min, freeze in MeOH/dry ice. Remove the organic layer and add it to 100 μL 0.5% phosphoric acid, vortex for 15 s, freeze in MeOH/dry ice. Discard the organic layer, remove the last traces of ether with nitrogen for 2-3 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 5 mm CN Microbond pak radial compression (Waters)
Mobile phase: MeCN:buffer 28:72 (Buffer was 75 mM phosphate and 20 mM dibutylamine, adjusted to pH 3.2.)
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5
Internal standard: antazoline hydrochloride (6.3)
Limit of detection: 2 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Simons,F.E.R.; Frith,E.M.; Simons,K.J. The pharmacokinetics and antihistaminic effects of brompheniramine, *J.Allerg.Clin.Immunol.*, **1982**, *70*, 458–464.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 226

CHROMATOGRAM

Retention time: 7.78
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for

5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 10.76
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: doxepin, methadone

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.935

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell incubations
Sample preparation: 40 mL Cell incubation + 50 mL MeOH, shake vigorously for 1 min, centrifuge at 2000 rpm for 10 min, wash the pellet twice with 50 mL portions of MeOH. Combine the supernatants and add 100 mL water, extract three times with 150 mL portions of dichloromethane. Filter the extracts through anhydrous sodium sulfate, evaporate the filtrate to dryness under reduced pressure at 40°, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano-propyl (Beckman)
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM triethyamine, pH 7.0.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Extracted: metabolites
Interfering: chlorpheniramine, pheniramine

KEY WORDS
also semipreparative details

REFERENCE
Hansen,E.B.,Jr.; Cho,B.P.; Korfmacher,W.A.; Cerniglia,C.E. Fungal transformations of antihistamines: metabolism of brompheniramine, chlorpheniramine, and pheniramine to *N*-oxide and *N*-demethylated metabolites by the fungus *Cunninghamella elegans*, *Xenobiotica*, **1995**, *25*, 1081–1092.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add N,N-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 μL aliquot of the supernatant. Urine. Inject a 100 μL aliquot of urine directly.

HPLC VARIABLES
Column: 150 × 4.6 Asahipak ODP-50 C18
Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 27:73
Flow rate: 0.8
Injection volume: 20-100

Detector: Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 8
Internal standard: N,N-dimethylbenzylamine
Limit of detection: 140 ng/mL

OTHER SUBSTANCES
Simultaneous: pheniramine, chlorpheniramine, pyrilamine, diphenhydramine

KEY WORDS
tablets

REFERENCE
Holeman,J.A.; Danielson,N.D. Liquid chromatography of antihistamines using post-column tris(2, 2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, *679*, 277–284.

SAMPLE
Matrix: incubations
Sample preparation: Extract incubation mixture with four volumes of cold dichloromethane for 3 min, centrifuge at 1000 g for 5 min. Remove the organic layer and pass it through a nylon filter, evaporate the filtrate to dryness, reconstitute the residue in 500 μL MeOH, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm AXXIOM silica (Richard Scientific)
Mobile phase: MeCN:MeOH:60% aqueous perchloric acid 60:40:0.08
Flow rate: 1
Injection volume: 15
Detector: UV 260

CHROMATOGRAM
Retention time: 6.4
Limit of detection: 5-20 ng/mL

OTHER SUBSTANCES
Extracted: chlorpheniramine N-oxide, chlorpheniramine N-oxide
Interfering: brompheniramine

KEY WORDS
ion-pair; desorption; chromatography; incubations

REFERENCE
Cashman,J.R.; Yang,Z.-C. Analysis of amine metabolites by high-performance liquid chromatography on silica gel with a non-aqueous ionic eluent, *J.Chromatogr.*, **1990**, *532*, 405–410.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)

Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 300 mM KCl and 300 mM ammonium chloride
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 265

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen,

procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: thonzylamine, pheniramine, tripelennamine, chlorpheniramine, phenindamine, phenyltoxamine, clemizole

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Inject 5 mg ±brompheniramine maleate onto analytical system. After separation on the analytical column use valve switching to collect the enantiomers on two 100 × 10 recovery columns of 12-20 μm Hamilton PRP-1 styrene-divinylbenzene columns. When sufficient material has been collected (60 mg), flush the recovery columns with 180 mL MeOH:water 5:95, and elute the enantiomers with MeOH (analytical column out of circuit). Each enantiomer can be purified by a second pass through the system.

HPLC VARIABLES

Column: 250 × 10 5 μm Spherisorb S5CN cyanopropyl

Mobile phase: MeOH:buffer 5:95 containing 12 mg/mL β-cyclodextrin hydrate (Buffer was 0.85% triethylamine adjusted to pH 4 with acetic acid.)

Flow rate: 3.5

Injection volume: 100

Detector: UV 285

CHROMATOGRAM

Retention time: 5.5 (+), 6.5 (-)

KEY WORDS
semi-preparative; chiral

REFERENCE
Cooper,A.D.; Jefferies,T.M. Semi-preparative high-performance liquid chromatographic resolution of brompheniramine enantiomers using β-cyclodextrin in the mobile phase, *J.Chromatogr.*, **1993**, *637*, 137–143.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, re-

serpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 60:35:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 12, 13 (enantiomers)

OTHER SUBSTANCES

Simultaneous: promethazine

KEY WORDS

chiral

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.10 (A), 5.44 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepox-

ide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

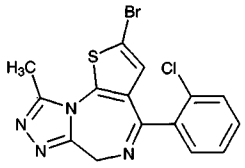
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Brotizolam



Molecular formula: $C_{15}H_{10}BrClN_4S$
Molecular weight: 393.69
CAS Registry No.: 57801-81-7
Merck Index: 1472
Lednicer No.: 4 219

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Urine, bile. Lyophilize, dissolve in 100 mM pH 5 sodium/potassium buffer, incubate with β-glucuronidase/sulfatase at 37° for 16 h, extract with ether. Plasma. Lyophilize, extract with MeOH.

HPLC VARIABLES

Guard column: 50 × 4.6 30-40 μm Lichrosorb RP18
Column: 250 × 4.6 10 μm Lichrosorb RP18
Mobile phase: MeCN:10 mM ammonium carbamate:diethylamine 35:65:0.1 (urine, bile) or MeOH:10 mM ammonium carbamate:diethylamine 45:55:0.1 (plasma)
Detector: UV 254

CHROMATOGRAM

Retention time: 23 (urine, bile), 39 (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; mouse; pharmacokinetics

REFERENCE

Bechtel,W.D. Blood level, metabolism and excretion of [^{14}C]-brotizolam in mice, *Eur.J.Drug Metab.Pharmacokinet.*, **1990**, *15*, 287–293.

SAMPLE

Matrix: blood, urine
Sample preparation: Wash a C2 Bond-Elut SPE cartridge with 1 column volume methanol and 1 column volume buffer. Add 1 mL of urine buffered with pH 6 100 mM phosphate buffer or plasma buffered with pH 8 100 mM phosphate buffer to the SPE cartridge, wash with 3 column volumes of water, wash with 1 mL of MeOH:water 30:70, elute with 1 mL of MeOH:water 60:40. Evaporate the eluate to dryness and take up the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 35 × 4.6 5 μm ultrabase C18 (Scharlau)
Mobile phase: MeOH:water 60:40
Flow rate: 0.9
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Internal standard: Prazepam
Limit of detection: 76 ng/mL

OTHER SUBSTANCES
Also analyzed: diazepam, oxazepam, nordazepam, temazepam, adinazolam, midazolam

KEY WORDS
plasma; SPE.

REFERENCE
Casas,M.; Berrueta,L.A.; Gallo,B.; Vicente,F. Solid-phase extraction of 1,4-benzodiazepines from biological fluids, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 277–284.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Mix 200 μL microsomal incubation with 5 μL 70% perchloric acid, cool on ice, add 5 μL 100 μM diazepam, centrifuge at 15000 g for 2 min. Transfer 170 μL supernatant to another tube and mix with 50 μL 1 M MOPS and 5 μL saturated KOH to adjust pH 7.0, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm Superspher RP-Select B (Merck)
Mobile phase: MeOH:MeCN:50mM ammonium acetate 50:5:45
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 5.2
Internal standard: diazepam (7.2)
Limit of quantitation: 100 nM

OTHER SUBSTANCES
Extracted: metabolites, α-hydroxy-brotizolam, 6-hydroxy-brotizolam KW human, liver

REFERENCE
Senda,C.; Kishimoto,W.; Sakai,K.; Nagakura,A.; Igarashi,T. Identification of human cytochrome P450 isoforms involved in the metabolism of brotizolam, *Xenobiotica*, **1997**, *27*, 913–922.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.43 (A), 7.90 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

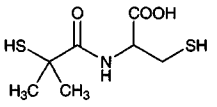
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Bucillamine



Molecular formula: $C_7H_{13}NO_3S_2$
Molecular weight: 223.32
CAS Registry No.: 65002-17-7
Merck Index: 1481

SAMPLE

Matrix: solutions
Sample preparation: Add 1.05-3 equivalents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-isothiocyanate to 10 mL of a 100 μM solution of the thiol in MeCN:water 50:50 containing 1-3 equivalents triethylamine, vortex briefly, let stand at room temperature for 30 min, dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)
Mobile phase: MeCN:10 mM pH 2.8 potassium phosphate buffer 53:47
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM

Retention time: 3.50 (S), 4.07 (R)

OTHER SUBSTANCES

Simultaneous: cysteine, homocysteine

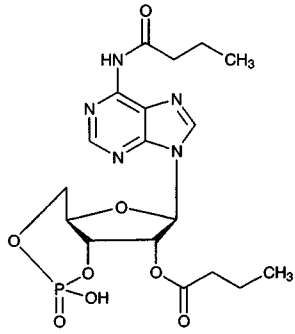
KEY WORDS

derivatization; chiral

REFERENCE

Ito,S.; Ota,A.; Yamamoto,K.; Kawashima,Y. Resolution of the enantiomers of thiol compounds by reversed-phase liquid chromatography using chiral derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, *J.Chromatogr.*, **1992**, *626*, 187–196.

Bucladesine



Molecular formula: $C_{18}H_{24}N_5O_8P$
Molecular weight: 469.39
CAS Registry No.: 362-74-3
Merck Index: 1483

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 10% trichloroacetic acid, vortex, cool in ice for 10 min, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:100 mM KH_2PO_4:water 13:2:85
Flow rate: 1
Detector: UV 273

CHROMATOGRAM

Limit of detection: <500 ng/mL

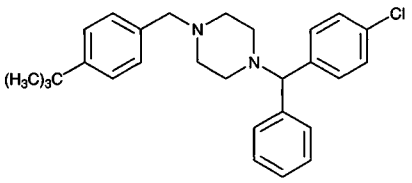
KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Mafune,E.; Takahashi,M.; Takasugi,N. Effect of vehicles on percutaneous absorption of bucladesine (dibutyryl cyclic AMP) in normal and damaged rat skin, *Biol.Pharm.Bull.*, **1995**, *18*, 1539–1543.

Buclizine



Molecular formula: $C_{28}H_{33}ClN_2$
Molecular weight: 433.04
CAS Registry No.: 82-95-1, 129-74-8 (HCl)
Merck Index: 1484

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 22.752

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 1.65

OTHER SUBSTANCES
Simultaneous: benactyzine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Schaeffer,V.H.; Masoud,A.N.; Rubin,R.J. Analysis of monobutyryl and dibutyryl derivatives of adenosine 3',5'-monophosphate in biological samples using isocratic ion pair high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 1255–1259.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pi-

zotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 1.65

OTHER SUBSTANCES

Simultaneous: benactyzine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 19

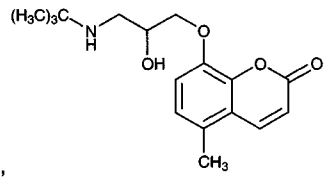
OTHER SUBSTANCES

Simultaneous: chlorcyclizine, tripelennamine, triprolidine, methaphenilene, pyrrobutamine, cyclizine, meclizine

REFERENCE

Vydac HPLC Catalog, 1994-5,

Bucumolol



Molecular formula: $C_{17}H_{23}NO_4$

Molecular weight: 305.37

CAS Registry No.: 58409-59-9, 80750-21-6 ((±)-form), 30073-40-6 (HCl), 36556-75-9 (HCl (±)-form)

Merck Index: 1489

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 µL of a 10 µM solution in MeCN:water:triethylamine 50:50:0.1 with 100 µL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 60F_{254} TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmeth-

yl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 37:63:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 37.2, 40.0 (enantiomers)
Limit of detection: 0.00303-0.00328 fmole

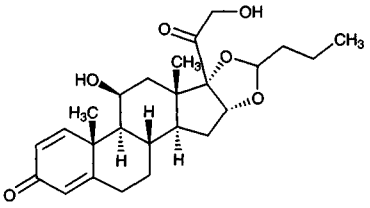
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Budesonide



Molecular formula: $C_{25}H_{34}O_6$
Molecular weight: 430.54
CAS Registry No.: 51333-22-3 (β,16α), 51372-29-3 (11β,16α[R]), 51372-28-2 (11β, 16α[S])
Merck Index: 1490
Lednicer No.: 3 95

SAMPLE

Matrix: blood
Sample preparation: Add 5 mL diethyl ether/dichloromethane (ratio not given) to 1 mL serum, mix for 30 min. Centrifuge and freeze at -80° for 15 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue in 200 µL mobile phase. Inject a 50 µL aliquot.

HPLC VARIABLES

Column: Lichrospher RP Select B
Mobile phase: MeCN:20 mM ammonium acetate buffer 80:20
Flow rate: 1
Injection volume: 50
Detector: MS, PE-Sciex API 300, negative ion mode

CHROMATOGRAM

Internal standard: budesonide

OTHER SUBSTANCES

Extracted: flunisolide

KEY WORDS

serum; budesonide is IS

REFERENCE

Möllmann,H.; Derendorf,H.; Barth,J.; Meibohm,B.; Wagner,M.; Krieg,M.; Weisser,H.; Knöller,J.; Möllmann,A.; Hochhaus,G. Pharmacokinetic/pharmacodynamic evaluation of systemic effects of flunisolide after inhalation, *J.Clin.Pharmacol.*, **1997**, *37*, 893–903.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge twice with 3 mL portions of EtOH and twice with 3 mL portions of water. Add 1 mL EtOH:water 30:70 to 1 mL plasma, vortex, let stand for 15 min. Centrifuge at 1800 g for 15 min. Add the supernatant dropwise to the SPE cartridge, wash with 3 mL EtOH:water 25:75, 3 mL water, and twice with 2 mL ethyl acetate:n-heptane 2:98. Dry the SPE cartridge under vacuum, elute with 2 mL ethyl acetate:heptane 35:65. Evaporate the eluate to dryness under a stream of nitrogen at 35°. Reconstitute the residue with 100 µL mobile phase, let stand 15 min. Inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 2.1 5 µm Hypersil ODS C18
Column: 100 × 2.1 5 µm Hypersil ODS
Mobile phase: EtOH:water 50:50
Flow rate: 0.45
Injection volume: 20
Detector: MS, Finnigan Mat TSQ 7000, ESI mode, positive ion APCI mode, m/z 473.2

CHROMATOGRAM
Retention time: 3.35 (budesonide acetate 22R epimer)
Internal standard: budesonide acetate 22R epimer

OTHER SUBSTANCES
Extracted: fluticasone

KEY WORDS
budesonide is IS; plasma; SPE

REFERENCE
Li,Y.N.; Tattam,B.N.; Brown,K.F.; Seale,J.P. A sensitive method for the quantification of fluticasone propionate in human plasma by high-performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 447–452.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg Extract Clean C18 SPE cartridge (Alltech) with two 3 mL portions of EtOH and two 3 mL portions of water. Add 50 μL 200 ng/mL IS solution and 1 mL EtOH:water 30:70 to 1 mL plasma, vortex carefully, let stand for 15 min, centrifuge at 1200 g for 20 min. Add the supernatant to the SPE cartridge attached to a vacuum manifold operating at 5.1 kPa, wash with 3 mL EtOH:water 25:75, 3 mL water, and 2 mL heptane:ethyl acetate 98:2. Elute with 2 mL heptane:ethyl acetate 65:35 and with 2 mL EtOH, evaporate the eluate to dryness under a stream of nitrogen at 35°, add 100 μL MeCN:triethylamine:acetic anhydride 75:12.5:12.5 to the residue, let react for 15 min, evaporate to dryness under a stream of nitrogen, reconstitute the residue with 100 μL mobile phase, let stand for at least 15 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm ODS Hypersil C18
Column: 100 × 2.1 5 μm ODS Hypersil C18
Mobile phase: EtOH:water 43:57
Flow rate: 0.5
Injection volume: 20
Detector: MS, Finnigan MAT TSQ 7000, APCI source, m/z 431, 476

CHROMATOGRAM
Retention time: 8.0 (22R), 9.0 (22S)
Internal standard: $^{2}H_{3}$ budesonide-21-acetate (preparation described in paper) (9-10.5)
Limit of detection: 700fg
Limit of quantitation: 250 pg/mL

KEY WORDS
derivatization; plasma; pharmacokinetics; SPE

REFERENCE
Li,Y.N.; Tattam,B.; Brown,K.F.; Seale,J.P. Determination of epimers 22R and 22S of budesonide in human plasma by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1996**, *683*, 259–268.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1.5 mL water + 4 mL dichloromethane, shake gently for 30 min, centrifuge at 2000 rpm for 15 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.9 10 μm Spherisorb ODS
Mobile phase: EtOH:water 40:60
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 7

KEY WORDS
plasma; dog; radiolabeled; pharmacokinetics

REFERENCE
Ryrfeldt,Å.; Tönnesson,M.; Nilsson,E.; Wikby,A. Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by high-performance liquid chromatography, *J.Steroid Biochem.*, **1979**, *10*, 317–324.

SAMPLE
Matrix: blood
Sample preparation: 250-500 μL blood + 10 mL EtOH:10 mM acetic acid 10:90, mix, add 9 mL to a conditioned Sep-Pak C18 SPE cartridge, wash with 10 mL EtOH:10 mM acetic acid 8:92, elute with 4 mL EtOH:10 mM acetic acid 60:40. Dilute the eluate to an EtOH content of 8% and add it to another conditioned C18 SPE cartridge, wash with 10 mL EtOH:10 mM acetic acid 8:92, elute with 4 mL EtOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 5 μm Nucleosil NO2 + 200 × 5 5 μm Nucleosil NO2 in series
Mobile phase: dichloromethane:isopropanol:water 99:1:0.2 for 35 min then 75:25:0.2 for 15 min, re-equilibrate at initial conditions for 45 min
Flow rate: 3
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 22-28

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; pharmacokinetics; SPE

REFERENCE
Andersson,P.; Appelgren,L.-E.; Ryrfeldt,Å. Tissue distribution and fate of budesonide in the mouse, *Acta Pharmacol.Toxicol.(Copenh)*, **1986**, *59*, 392–402.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C18 SPE cartridge. Add plasma + IS to SPE cartridge, wash with aqueous EtOH, wash with water, wash with heptane, elute with ethyl acetate in heptane, esterify with acetic anhydride and triethylamine in MeCN, evaporate, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chromguard
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:100 mM pH 5 ammonium acetate 64:36
Flow rate: 1.4
Injection volume: 100
Detector: MS, Finnigan 4500 quadrupole, thermospray, scan time 40 ms, source block 220°, repeller 45 V, vaporizer 105°, jet block 180°, aerosol 220

CHROMATOGRAM
Retention time: 3.6 (as budesonide 21-acetate)
Internal standard: octadeutero budesonide
Limit of quantitation: 0.1 nM

KEY WORDS
plasma; LC-MS; SPE; derivatization

REFERENCE
Lindberg,C.; Paulson,J.; Blomqvist,A. Evaluation of an automated thermospray liquid chromatography-mass spectrometry system for quantitative use in bioanalytical chemistry, *J.Chromatogr.*, **1991**, *554*, 215–226.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2500 g for 10 min, mix the supernatant with an equal volume of 1 M pH 2.5 glycine buffer containing 0.2% Tween 20, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot of the supernatant on to column A and elute to waste with mobile phase A, after 1.4 min backflush the contents of column A on to column B with mobile phase B, after 3.6 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Flush column A with mobile phase A for 9 min and mobile phase B for 16 min.

HPLC VARIABLES
Column: A 30 × 2.1 Apex II aminopropyl (Jones Chromatography); B 50 × 2.1 Spherisorb C1 pH stable
Mobile phase: A 10 mM pH 2.5 glycine buffer containing 0.1% Tween 20; B Isopropanol: 10 mM pH 2.5 glycine buffer containing 0.1% Tween 20 3:97
Column temperature: 40
Flow rate: 0.5
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 22, 25 (epimers)

KEY WORDS
plasma; column-switching

REFERENCE
Lövgren,U.; Johansson,M.; Kronkvist,K.; Edholm,L.-E. Biocompatible sample pretreatment for immunochemical techniques using micellar liquid chromatography for separation of corticosteroids, *J.Chromatogr.B*, **1995**, *672*, 33–44.

SAMPLE
Matrix: blood, tissue
Sample preparation: Acidify plasma or lung tissue homogenate to pH 2 with 500 mM HCl, add 100 μL 20 μg/mL IS, extract with 8 mL dichloromethane. Evaporate the organic

layer to dryness under vacuum, reconstitute in 120 μL MeOH:5% acetic acid 50:50, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS C18
Mobile phase: MeCN:MeOH:water 44:11:45
Flow rate: 1
Injection volume: 80
Detector: UV 242 or radioactivity

CHROMATOGRAM
Internal standard: hydrocortisone 21-S-propionate (JO 498)

OTHER SUBSTANCES
Extracted: metabolites, beclomethasone dipropionate

KEY WORDS
plasma; rat; lung; radiolabeled; pharmacokinetics; epimers are separated

REFERENCE
Chanoine,F.; Grenot,C.; Heidmann,P.; Junien,J.L. Pharmacokinetics of butixocort 21-propionate, budesonide, and beclomethasone dipropionate in the rat after intratracheal, intravenous, and oral treatments, *Drug Metab.Dispos.*, **1991**, *19*, 546–553.

SAMPLE
Matrix: broncho-alveolar lavage fluid
Sample preparation: Centrifuge bronchoalveolar lavage fluid at 1150 g for 10 min. 1 mL Supernatant + 5 mL dichloromethane, shake on an alternating agitator for 10 min, centrifuge at 1150 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, centrifuge, inject an 80 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS C18
Mobile phase: MeOH:buffer 69:31 (Buffer was 0.1% acetic acid, pH 3.)
Flow rate: 1
Injection volume: 80
Detector: UV 250

CHROMATOGRAM
Retention time: 10.51
Limit of quantitation: 5 ng/mL

REFERENCE
Faouzi,M.A.; Dine,T.; Luyckx,M.; Brunet,C.; Gressier,B.; Cazin,M.; Wallaert,B. High-performance liquid chromatographic method for the determination of budesonide in bronchoalveolar lavage of asthmatic patients, *J.Chromatogr.B*, **1995**, *664*, 463–467.

SAMPLE
Matrix: bulk
Sample preparation: Inject a 5 μL aliquot of a solution in EtOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: EtOH:water 48:52 or 43:57
Flow rate: 1
Injection volume: 5

Detector: UV 254

CHROMATOGRAM
Retention time: 10, 11 (48:52), 18, 25 (43:57) (two epimers)

OTHER SUBSTANCES
Simultaneous: impurities, 16α-hydroxyprednisolone

REFERENCE
Roth,G.; Wikby,A.; Nilsson,L.; Thalén,A. High-performance liquid chromatographic determination of epimers, impurities, and content of the glucocorticoid budesonide and preparation of primary standard, *J.Pharm.Sci.*, **1980**, *69*, 766–770.

SAMPLE
Matrix: solutions
Sample preparation: Mix 250 µL of a solution of budesonide in MeOH with 625 µL 9% trifluoromethanesulfonic acid in MeOH, immediately add 625 µL 160 µm dansyl hydrazine in MeOH, after 12 h inject a 100 µL aliquot onto column A and elute to waste with mobile phase A, after 100 s backflush the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. (Caution! Trifluoromethanesulfonic acid is highly toxic! Decontaminate surplus solutions with an equal volume of 2 M aqueous ammonia for 1 h!)

HPLC VARIABLES
Column: A 10 × 4.6 5 µm Vydac C18; B 150 × 4.6 5 µm Nucleosil C18
Mobile phase: A MeCN:7.7 mM pH 7 phosphate buffer 6:94; B MeCN:7.7 mM pH 7 phosphate buffer 65:35
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 520

CHROMATOGRAM
Retention time: 8, 10 (syn and anti isomers of 3-keto derivative)
Limit of detection: 1.5 pmole

KEY WORDS
derivatization; column-switching

REFERENCE
Hyytiäinen,M.; Appelblad,P.; Pontén,E.; Stigbrand,M.; Irgum,K.; Jaegfeldt,H. Trifluoromethanesulfonic acid as a catalyst for the formation of dansylhydrazone derivatives, *J.Chromatogr.A*, **1996**, *740*, 279–283.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize tissue in ice cold saline for 30 s. 100 µL Homogenate + 4 mL EtOH:10 mM acetic acid 70:30, shake for 30 min, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 µL EtOH:water 30:70, add 5 mL 10 mM acetic acid, add to a conditioned Sep-Pak C18 SPE cartridge, wash with 5 mL 10 mM acetic acid, elute with 5 mL EtOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute in 250 µL EtOH:water 30:70, inject a 200 µL aliquot.

HPLC VARIABLES
Column: 250 × 5 5 µm Nucleosil C18
Mobile phase: EtOH:water 50:50 for 12 min then EtOH for 10 min
Flow rate: 1

Injection volume: 200
Detector: UV 254

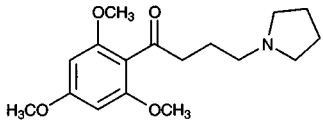
CHROMATOGRAM
Retention time: 11-13

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; pharmacokinetics; liver; lung; spleen; brain; SPE

REFERENCE
Andersson,P.; Appelgren,L.-E.; Ryrfeldt,Å. Tissue distribution and fate of budesonide in the mouse, *Acta Pharmacol.Toxicol.(Copenh)*, **1986**, *59*, 392–402.

Buflomedil



Molecular formula: $C_{17}H_{25}NO_4$
Molecular weight: 307.39
CAS Registry No.: 55837-25-7, 35543-24-9 (HCl)
Merck Index: 1498

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM

Retention time: 11.3

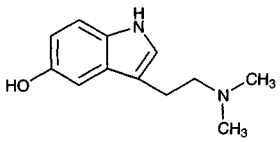
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bufotenine



Molecular formula: $C_{12}H_{16}N_2O$
Molecular weight: 204.27
CAS Registry No.: 487-93-4
Merck Index: 1502

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

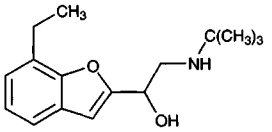
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine,

quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Bufuralol



Molecular formula: $C_{16}H_{23}NO_2$, $C_{16}H_{23}NO_2$.HCl (hydrochloride)
Molecular weight: 261.36
CAS Registry No.: 54340-62-4, 59652-29-8 (HCl), 57704-10-6 ((-), HCl), 57704-11-7 ((+), HCl)
Merck Index: 1504

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 500 µL immobilized antibody (details of preparation in paper), wash with 5 mL 1 M NaCl, wash with 10 mL water, elute with 5 mL MeOH:10 mM pH 5.0 ammonium acetate buffer 95:5, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Ultron ES-OVM (Shinwa, Osaka, Japan)
Mobile phase: MeCN:0.3% pH 6.7 ammonium acetate buffer from 1:14 to 1:2 over 40 (?) min
Flow rate: 1
Detector: UV 248

CHROMATOGRAM

Retention time: 32

OTHER SUBSTANCES

Extracted: metabolites

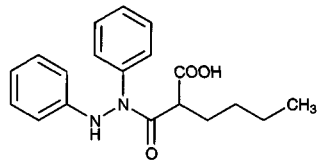
KEY WORDS

plasma; chiral separation of the metabolites but not necessarily of bufuralol

REFERENCE

Ikegawa,S.; Matsuura,K.; Sato,T.; Made,N.; Isriyanthi,R.; Niwa,T.; Miyairi,S.; Takashima,H.; Kawashima,Y.; Goto,J. Enantioselective immunoaffinity extraction for simultaneous determination of optically active bufuralol and its metabolites in human plasma by HPLC, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 1–9.

Bumadizon



Molecular formula: $C_{19}H_{22}N_2O_3$
Molecular weight: 326.40
CAS Registry No.: 3583-64-0, 69365-73-7 (calcium salt hemihydrate)
Merck Index: 1507

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 235

CHROMATOGRAM

Retention time: 6.72
Limit of detection: <120 ng/mL

KEY WORDS

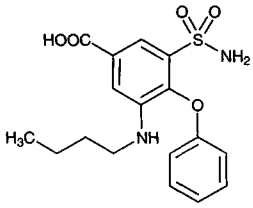
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Bumetanide



Molecular formula: $C_{17}H_{20}N_2O_5S$
Molecular weight: 364.42
CAS Registry No.: 28395-03-1
Merck Index: 1508
Lednicer No.: 2 87

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 30 µL 500 ng/mL methylbumetanide in water + 200 µL 1 M sulfuric acid + 6 mL diethyl ether, shake 20 min, centrifuge at 1000 g for 5 min. Evaporate 4 mL of ether layer at 30° under a stream of nitrogen. Dissolve residue in 150 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 22 × 3.9 37-50 µm Corasil C18
Column: 75 × 4.6 3 µm Supelco LC-8-DB
Mobile phase: MeCN:30 mM pH 3.0 sodium phosphate buffer 125:200
Flow rate: 1
Injection volume: 100
Detector: F ex 340 em 440

CHROMATOGRAM

Retention time: 4.5
Internal standard: methylbumetanide (6.5)
Limit of detection: 0.1 ng/mL

KEY WORDS

plasma

REFERENCE

Bökens,H.; Bourscheidt,C.; Müller,R.F. Determination of bumetanide in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *434*, 327–329.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 500 ng naproxen + 1 mL 100 mM HCl + 10 mL dichloromethane, extract. Dry organic layer at 50° under nitrogen, dissolve in 1 mL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 µm Alltech C8
Mobile phase: MeCN:80 mM phosphoric acid 35:65
Flow rate: 1
Injection volume: 20
Detector: F ex 235 em 405

CHROMATOGRAM

Retention time: 12.3
Internal standard: naproxen (3.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: furosemide

KEY WORDS
plasma; horse; pharmacokinetics

REFERENCE
Singh,A.K.; McArdle,C.; Gordon,B.; Ashraf,M.; Granley,K. Simultaneous analysis of furosemide and bumetanide in horse plasma using high performance liquid chromatography, *Biomed.Chromatogr.*, **1989**, *3*, 262–265.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + piretanide + 1 mL MTBE + 100 μL 1 M pH 4 phosphate buffer, extract for 5 min, centrifuge. Remove 850 μL of the organic layer and evaporate it to dryness, reconstitute the residue in 180 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:50 mM pH 3 NaH_2PO_4 30:70
Flow rate: 2
Injection volume: 50
Detector: F (wavelengths not specified)

CHROMATOGRAM
Retention time: 6.2
Internal standard: piretanide (3.9)
Limit of detection: 0.4 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Oberbauer,R.; Krivanek,P.; Turnheim,K. Pharmacokinetics and pharmacodynamics of the diuretic bumetanide in the elderly, *Clin.Pharmacol.Ther.*, **1995**, *57*, 42–51.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 50 μL 250 μg/mL acetophenone in MeCN: water 50:50, vortex, add 400 μL MeCN, vortex, sonicate for 2 min, centrifuge for 10 min, inject an aliquot of the supernatant. Tissue. Homogenize (Tissuemizer) rat stomach, kidney, or liver with three volumes cold 250 mM sucrose, centrifuge at 9000 g. Incubate 1 mL supernatant with bumetanide, add 1 mL 1 M NaOH. 100 μL Mixture + 250 μL MeCN + 100 μL 0.3% HCl, vortex, centrifuge, inject an aliquot of the supernatant (from Biopharm. Drug Dispos. 1991, 12, 311).

HPLC VARIABLES
Column: 250 × 4.6 Partisil-10 ODS-3
Mobile phase: MeOH:water:acetic acid 70:30:1
Flow rate: 1.5
Detector: F ex 338 em 433 or UV 254

CHROMATOGRAM
Retention time: 5.5 (F)
Internal standard: acetophenone (4.0, UV)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; human; rat; pharmacokinetics; stomach; kidney; liver; rabbit (J.Pharm.Sci. 1995; 84; 236)

REFERENCE
Smith,D.E. High-performance liquid chromatographic assay for bumetanide in plasma and urine, *J.Pharm.Sci.*, **1982**, *71*, 520–523.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 400 μL Serum + 100 μL 500 ng/mL IS + 1.0 mL 1.0 M pH 5.0 potassium citrate, vortex, let sit for 1 min. Add 4.0 mL ethyl acetate:cyclohexane 70:30, mix (Rototorque mixer) at 60 rpm for 10 min, centrifuge at 1150 g for 1 min. Evaporate upper organic layer to dryness under reduced pressure. Redissolve residue in 125 μL mobile phase, inject a 50 μL aliquot. Urine. Centrifuge sample at 16000 g for 1 min. Mix one volume urine supernatant with one volume 800 mM pH 3.9 potassium formate buffer, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 Zorbax C8
Mobile phase: MeOH:30 mM pH 2.5 potassium phosphate 63:37
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 340 em 440

CHROMATOGRAM
Retention time: 6.8
Internal standard: R021-1825 (6.3)
Limit of quantitation: 3 ng/mL (plasma), 10 ng/mL (urine)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Sullivan,J.E.; Witte,M.K.; Yamashita,T.S.; Myers,C.M.; Blumer,J.L. Pharmacokinetics of bumetanide in critically ill infants, *Clin.Pharmacol.Ther.*, **1996**, *60*, 405–413.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond Elut 1 mL C18 SPE cartridge with 600 μL MeCN, 2 mL MeOH, and 3 mL water. 200 μL Plasma or urine + 20 (plasma) or 100 (urine) ng piretanide in methanol + 400 μL MeCN, vortex 2 min, sonicate 2 min in a water bath, centrifuge 10 min 1000 g. Transfer supernatant to a clean tube and add 4 mL 100 mM pH 5.0 phosphate buffer, add to SPE cartridge, wash with 3 mL water, elute with 300 μL MeCN. Evaporate to dryness under a stream of nitrogen at 37°, take up in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: C18 (unspecified)
Column: 100 × 8 5 μm C18 Radial Pak
Mobile phase: MeOH:water:glacial acetic acid 66:34:1
Flow rate: 1.2
Injection volume: 100
Detector: F ex 228 em 418

CHROMATOGRAM
Retention time: 6.5

Internal standard: piretanide (5.1)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: dopamine, amikacin, furosemide
Noninterfering: carbamazepine, phenytoin, midazolam, diazepam, lorazepam, ranitidine, gentamicin, erythromycin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, vancomycin, clindamycin, theophylline, digoxin
Interfering: nafcillin

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Wells,T.G.; Hendry,I.R.; Kearns,G.L. Measurement of bumetanide in plasma and urine by high-performance liquid chromatography and application to bumetanide disposition, *J.Chromatogr.*, **1991**, *570*, 235–242.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 19.055

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in mobile phase:acetone 95:5 containing 100 μg/mL salicylic acid, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Varian RP-8
Mobile phase: MeOH:water:acetic acid 60:40:0.5
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: 9.3
Internal standard: salicylic acid (4.5)
Limit of detection: 2000 ng/mL

KEY WORDS
tablets; ampoules

REFERENCE
Zivanov-Stakic,D.; Solomun,L.J.; Zivanovic,L.J. High-performance liquid chromatographic method for the determination of bumetanide in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1889–1892.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 254, F ex 228 em 418

CHROMATOGRAM
Retention time: 8.9
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: amiloride, furosemide, triamterene

KEY WORDS
column-switching; pharmacokinetics

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 100 μL water + 50 μL 500 μg/mL acetophenone in MeCN:water 50:50, vortex, inject an aliquot. (For rabbit (J.Pharm.Sci. 1995, 84, 236) or

rat (Biopharm. Drug Dispos. 1991, 12, 311) 100 μL urine + 250 μL MeCN, vortex, centrifuge, inject an aliquot.)

HPLC VARIABLES
Column: 250 × 4.6 Partisil-10 ODS-3
Mobile phase: MeCN:15 mM phosphoric acid 50:50
Flow rate: 2
Injection volume: 6.5 (F)
Detector: F ex 338 em 433 or UV 254

CHROMATOGRAM
Internal standard: acetophenone (4.5, UV)

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
pharmacokinetics; human; rabbit; rat

REFERENCE
Smith,D.E. High-performance liquid chromatographic assay for bumetanide in plasma and urine, *J.Pharm.Sci.*, **1982**, *71*, 520–523.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM
Retention time: 9.3
Limit of quantitation: 1500 ng/mL

OTHER SUBSTANCES
Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, chlorthalidone, clopamide, methyclothiazide, furosemide, metolazone, mefruside, cyclopenthiazide, bendroflumethiazide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE
Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 15.25 (A), 15.8 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

Interfering: ethacrynic acid

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 1 mL 10 mM HCl + 2000 ng bendroflumethiazide, extract with 5 mL ethyl acetate, centrifuge at 3000 rpm for 5 min. Remove the organic layer and dry it under a stream of nitrogen at 40°. Reconstitute with 100 μL MeOH, inject a 2 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 μm Hypersil ODS

Mobile phase: Gradient. MeOH: 50 mM ammonium acetate from 10:90 to 60:40 over 10 min, maintain at 60:40 for 10 min.

Column temperature: 40

Flow rate: 0.3

Injection volume: 2

Detector: F ex 231 em 426 or UV 230

CHROMATOGRAM

Retention time: 6.2

Internal standard: Bendroflumethiazide (F ex 223 em 415) (8.6)
Limit of detection: <10 ng/mL

OTHER SUBSTANCES
Extracted: furosemide (UV), piretanide (UV), cyclopenthiazide (UV), etozolin (UV), canrenone (UV)

REFERENCE
Gradeen,C.Y.; Billay,D.M.; Chan,S.C. Analysis of bumetanide in human urine by high-performance liquid chromatography with fluorescence detection and gas chromatography/mass spectrometry, *J.Anal.Toxicol.*, **1990**, *14*, 123–126.

SAMPLE
Matrix: urine
Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)
Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5
Flow rate: 0.8 or 1
Injection volume: 10-20
Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 3.7
Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES
Extracted: probenecid, ethacrynic acid, spironolactone

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 19.5

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85, inject a 20 μL aliquot. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid from 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min, maintain at 60:40 for 1 min, decrease to 10:90 over 1 min, equilibrate at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 7.2

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

Interfering: xipamide

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Inject 50 μL urine directly onto column A with mobile phase A and elute to waste, after 1 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 10
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene
Interfering: ethacrynic acid

KEY WORDS

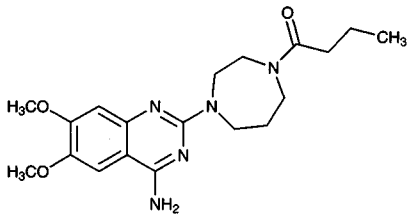
column-switching

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Bunazosin

Molecular formula: $C_{19}H_{27}N_5O_3$
Molecular weight: 373.46
CAS Registry No.: 80755-51-7
Merck Index: 1512



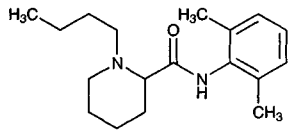
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:17 mM acetate containing 5 mM sodium laurylsulfate 50:50
Flow rate: 1
Detector: UV 245

KEY WORDS
water; buffer

REFERENCE
Kato,A.; Iwata,S. Studies on improved corneal permeability to bunazosin, *J.Pharmacobiodyn.*, **1988**, *11*, 330–334.

Bupivacaine



Molecular formula: $C_{18}H_{28}N_2O$
Molecular weight: 288.43
CAS Registry No.: 2180-92-9, 14252-80-3 (HCl hydrate), 18010-40-7 (HCl)
Merck Index: 1520
Lednicer No.: 1 17

SAMPLE

Matrix: blood
Sample preparation: Mix 50 μL water with 500 μL pH 6.0 buffer and 100 μL plasma sample, add 10 mL chloroform, shake mechanically for 10 min, centrifuge at 873 g for 10 min, evaporate the organic layer under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 35 μL aliquot. (Prepare pH 6.0 buffer by mixing 67 mM KH_2PO_4 with 67 mM Na_2HPO_4 in an 87.7:12.3 ratio.)

HPLC VARIABLES

Column: 250 × 2.1 5μm Supelcosil ABZ+plus deactivated reversed-phase
Mobile phase: MeOH:MeCN:50 mM pH 4.5 monobasic ammonium phosphate 5:7:63
Flow rate: 0.4
Injection volume: 35
Detector: UV 235

CHROMATOGRAM

Retention time: 26.90
Internal standard: bupivacaine (26.90)

OTHER SUBSTANCES

Extracted: cocaine, benzoylecgonine, norcocaine, cocaethylene,
Simultaneous: ascorbic acid, morphine, oxymorphone, noroxymorphone, norhydromorphone, norcodeine, codeine, nalorphine, procaine, acetaminophen, oxycodone, hydrocodone, caffeine, ethylmorphine, lidocaine, benzoylnorecgonine, ketamine, acepromazine, salicylic acid, benzoic acid, thebaine, cocaine propyl ester, benzocaine, tetracaine, pentobarbital

KEY WORDS

rat; plasma; pharmacokinetics; bupivacaine is IS

REFERENCE

Pan,W.; Hedaya,M.A. Sensitive and specific high-performance liquid chromatographic assay with ultraviolet detection for the determination of cocaine and its metabolites in rat plasma, *J.Chromatogr.B*, **1997**, *703*, 129–138.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 μL 2 μg/mL IS in MeOH, add 2 mL water and 2 mL MeCN, vortex gently, set aside for 3 min, centrifuge at 2200 g for 20 min. Separate the clear supernatant, add 500 μL 200 mM NaOH and extract with 6 mL n-hexane by vortexing for 2 min. Centrifuge at 2200 g for 15 min. Evaporate 5 mL of the organic phase to dryness under reduced pressure. Reconstitute the residue in 120 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 5 μm AGP bonded silica (ChromTech, Hagersten, Sweden)
Column: 150 × 4 5 micro.m AGP bonded silica (ChromTech, Hagersten, Sweden)

Mobile phase: Isopropanol:buffer 4:96 (Prepare mobile phase by adding 4% isopropanol and 0.6% diethylamine to 8 mM sodium dihydrogen phosphate containing 100 mM NaCl, adjust to pH 7.05 with 50% phosphoric acid.)
Flow rate: 0.9
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 29.35 (R-(+)), 38.25 (S-(-))
Internal standard: diazepam(19.21)
Limit of detection: 4 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine

KEY WORDS
plasma; pharmacokinetics; chiral

REFERENCE
Abraham,I.; Fawcett,J.P.; Kennedy,J.; Kumar,A.; Ledger,R. Simultaneous analysis of lignocaine and bupivacaine enantiomers in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *703*, 203–208.

SAMPLE
Matrix: blood
Sample preparation: Directly inject 100 μL serum onto column A and elute to waste with mobile phase A, after 2 min elute to waste with mobile phase B, after 2 min backflush the contents of column A onto column B with mobile phase C, after 2 min remove column A from the circuit, elute column B with mobile phase C, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 4 min.

HPLC VARIABLES
Column: A 20 × 4.6 protein-coated Lichrosorb RP-8 (preparation details not given); B 250 × 4.6 Lichrosorb RP-18
Mobile phase: A pH 7.4 phosphate buffered saline; B MeOH:100 mM pH 5.5 phosphate buffer 20:80; C MeCN:MeOH:pH 6.4 phosphate buffer:ethylamine 30:30:40:0.3
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: <10
Limit of detection: 70 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, codeine, epinephrine, lignocaine, meperidine, morphine

KEY WORDS
serum; column-switching

REFERENCE
Emara,S.; Khedr,A.; Askal,H. Rapid and specific precolumn extraction high-performance liquid chromatographic assay for bupivacaine in human serum, *Biomed.Chromatogr.*, **1996**, *10*, 131–134.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 50 μL 40 μg/mL etidocaine hydrochloride in water + 100 μL 1 M NaOH, vortex for 15 s, add 5 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.2 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 5.80 Na_2HPO_4 25:75
Flow rate: 0.9
Injection volume: 80
Detector: UV 210

CHROMATOGRAM

Retention time: 8.7
Internal standard: etidocaine (12.0)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: 2,6-pipecolylxylidine, mepivacaine, lidocaine
Noninterfering: metabolites, 2,3-chloroprocaine, theophylline, mexiletine, quinidine, disopyramide, verapamil, phenobarbital, phenytoin, carbamazepine, ethosuximide, digoxin, theobromine, caffeine, furosemide, phenprocoumon, aldactone

KEY WORDS

plasma

REFERENCE

Ha,H.-R.; Funk,B.; Gerber,H.R.; Follath,F. Determination of bupivacaine in plasma by high-performance liquid chromatography, *Anesth.Analg.*, **1984**, *63*, 448–450.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 μg diazepam +100 μL 2 M NaOH + 7 mL n-hexane, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 Enantiopak α1-acid glycoprotein (LKB)
Mobile phase: Isopropanol:8 mM sodium phosphate buffer containing 100 mM NaCl 9:91
Column temperature: 30
Flow rate: 0.3
Injection volume: 20
Detector: UV 215

CHROMATOGRAM

Retention time: 21.0 (R), 28.3 (S)
Internal standard: diazepam (15.7)
Limit of quantitation: 500 ng/mL

KEY WORDS

serum; chiral

REFERENCE

Lee,E.J.; Ang,S.B.; Lee,T.L. Stereoselective high-performance liquid chromatographic assay for bupivacaine enantiomers, *J.Chromatogr.*, **1987**, *420*, 203–206.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 2 μg/mL etidocaine in water + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 20:80, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 12
Internal standard: etidocaine (10)
Limit of detection: 2 ng/mL

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 100 μg/mL lidocaine hydrochloride in water + 100 μL 2 M NaOH, mix, add 3 mL n-hexane, shake for 1 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and evaporate it to dryness with nitrogen under vacuum, reconstitute the residue in 200 μL mobile phase, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:50 mM pH 5.9 KH_2PO_4 38:62
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 5.9
Internal standard: lidocaine (3.8)
Limit of detection: 25 ng/mL

KEY WORDS
plasma

REFERENCE
Murillo,I.; Costa,J.; Salvá,P. Determination of bupivacaine in human plasma by HPLC, *J.Liq.Chromatogr.*, **1993**, *16*, 3509–3514.

SAMPLE
Matrix: blood

Sample preparation: Condition a Bakerbond cyano SPE cartridge two 1 mL aliquots of eluent. Add 2 mL MeCN to 1 mL plasma slowly while whirlmixing, let stand at room temperature for 5 min, centrifuge at 3000 g for 10 min. Remove the supernatant and dilute it with 15 mL water, add this mixture to the SPE cartridge, wash with three 1 mL aliquots of water, elute with eluent. Evaporate the eluent to about 500 μL under a stream of nitrogen, add 1 mL water, add 50 μL 1 M NaOH, whirlmix for 30 s, add 6 mL n-hexane, rotate for 10 min, centrifuge at 3000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 80 μL mobile phase, let stand for 2 h, inject a 50 μL aliquot. (The eluent was MeOH:50 mM NaH_2PO_4 adjusted to pH 3.0 with 1 M phosphoric acid 50:50.)

HPLC VARIABLES

Guard column: 10 × 4 Bakerbond chiral α1-acid glycoprotein
Column: 100 × 4 Bakerbond chiral α1-acid glycoprotein
Mobile phase: Isopropanol:10 mM pH 6.8 NaH_2PO_4 6:94
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 20 (R-(+)), 25 (S-(-))
Limit of detection: 10 ng/mL (S), 8 ng/mL (R)

KEY WORDS

plasma; SPE; chiral; pharmacokinetics

REFERENCE

Groen,K.; Zeijlmans,P.W.M.; Burm,A.G.L.; van Kleef,J.W. Improved clean-up procedure for the high-performance liquid chromatographic assay of bupivacaine enantiomers in human plasma and ultrafiltrate in the nanogram per milliliter range, *J.Chromatogr.B*, **1994**, *655*, 163–166.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL IS solution and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Prepare IS solution by adding 40 μL 1 mg/mL N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) in MeOH to 10 mL 100 mM NaH_2PO_4. Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM

Retention time: 7.2
Internal standard: N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) (14.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, mepivacaine, meperidine, fentanyl
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 2 M NaOH, vortex briefly, add 5 mL anhydrous ethyl ether, vortex for 30 s, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove 4.5 mL ether and add to 250 μL 12.5 mM sulfuric acid, vortex for 30 s, rotate for 10 min, centrifuge for 5 min, inject a 50 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Octyl 1B (Keystone)
Mobile phase: MeCN:50 mM Na_2HPO_4 27:73 pH adjusted to 5.8 with 50% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 9.18
Internal standard: bupivacaine

OTHER SUBSTANCES
Extracted: lidocaine, prilocaine, o-toluidine

KEY WORDS
plasma; pig; bupivacaine is IS

REFERENCE
Klein,J.; Fernandes,D.; Gazarian,M.; Kent,G.; Koren,G. Simultaneous determination of lidocaine, prilocaine and the prilocaine metabolite o-toluidine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *655*, 83–88.

SAMPLE
Matrix: blood
Sample preparation: 450 μL Plasma + 50 μL pentycaine in 10 mM pH 7 ACES buffer, vortex, filter (0.22 μm nylon syringe filter), inject a 10 μL aliquot onto column A with mobile phase A, after 3 min elute contents of column A onto column B with mobile phase B, monitor the effluent from column B. After 3 min remove column A from the circuit and re-equilibrate with mobile phase A. (ACES is N-(2-acetamido)-2-aminoethanesulfonic acid.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Regis semi-permeable surface (SPS) guard cartridge; B 100 × 3.2 7 μm Hypercarb pH (Shandon)
Mobile phase: A Isopropanol:buffer 3:97 (Buffer was 10 mM ACES adjusted to pH 7.0 with 5 M ammonia solution. ACES is N-(2-acetamido)-2-aminoethanesulfonic acid.); B 10 mM Acetic acid and 4 mM triethylamine in MeOH
Flow rate: A 0.8; B 0.5
Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: pentycaine (6)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: ropivacaine

KEY WORDS
plasma; column switching

REFERENCE
Yu,Z.; Abdel-Rehim,M.; Westerlund,D. Determination of amide-type local anaesthetics by direct injection of plasma in a column-switching high-performance liquid chromatographic system using a pre-column with a semipermeable surface, *J.Chromatogr.B*, **1994**, *654*, 221–230.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of plasma to 7.4 with carbon dioxide gas. Filter (Amicon MPS-1 with YMT 30 membrane) while centrifuging at 37° at 500 g for 15 min, inject a 150 μL aliquot of the ultrafiltrate on to column A and elute to waste with mobile phase A, collect the eluate containing the compound in a 1 mL loop and inject it onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES
Column: A 125 × 4 4 μm Superspher RP-select B (Merck); B 150 × 4.6 5 μm Nucleosil 5 SA
Mobile phase: A MeCN:buffer 30:70 (Buffer was 3.1 mL 1 M phosphoric acid and 20 mL 1 M NaH_2PO_4 made up to 1 L with water, pH 3.); B MeCN:buffer 40:60 (Buffer was 149 mL 2 M ammonium hydroxide and 348 mL 1 M phosphoric acid made up to 1 L with water, pH 2.6.)
Column temperature: 27
Flow rate: 1
Injection volume: 150
Detector: UV 210

CHROMATOGRAM
Retention time: 12
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: ropivacaine (elutes in a different fraction from column A)

KEY WORDS
plasma; ultrafiltrate; column-switching; rugged

REFERENCE
Arvidsson,T.; Eklund,E. Determination of free concentration of ropivacaine and bupivacaine in blood plasma by ultrafiltration and coupled-column liquid chromatography, *J.Chromatogr.B*, **1995**, *668*, 91–98.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Clean Screen SPE cartridge (Worldwide Monitoring) with two 2 mL portions of MeOH, with 3 mL water, and with 3 mL 10 mM pH 3.0

phosphate buffer. 1 mL Serum + 500 μL 10 mM pH 3.0 phosphate buffer, mix, add to the SPE cartridge, air dry for 30 s, wash with 3 mL phosphate buffer, wash with 3 mL 100 mM HCl, wash with 3 mL MeOH, elute with 2 mL chloroform:isopropanol:ammonium hydroxide 22:20.5:2.5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 SemiPermeable Surface (SPS) C8 (Regis)
Mobile phase: THF:2.5 mM potassium phosphate buffer 3.25:96.75 containing 0.0025% triethylamine, final pH adjusted to 2.7-2.8 with 85% orthophosphoric acid
Flow rate: 0.5
Detector: UV 235

CHROMATOGRAM

Retention time: 24
Internal standard: bupivacaine
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: acepromazine, atropine, benzoylecgonine, benzoylnorecgonine, cocaine, ketamine, norcocaine
Noninterfering: benzethonium chloride, benzyl alcohol

KEY WORDS

SPE; serum; bupivacaine is IS

REFERENCE

Muztar,J.; Chari,G.; Bhat,R.; Ramaro,S.; Vidyasagar,D. A high-performance liquid chromatographic procedure for the separation of cocaine and some of its metabolites from acepromazine, ketamine, and atropine from serum, *J.Liq.Chromatogr.*, **1995**, *18*, 2635–2645.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 10 μg/mL lidocaine in 25 mM sulfuric acid + 1 mL 1 M NaOH + 5 mL diethyl ether, shake or rotate for 15 min, centrifuge at 1000 rpm for 5 min, freeze at -20°. Remove the organic layer and add it to 250 μL 25 mM sulfuric acid, shake for 15 min, centrifuge at 1000 rpm for 5 min, freeze, discard the organic layer. Thaw the aqueous layer, pass air over the aqueous phase at room temperature to remove traces of ether, adjust pH to 5.0-6.5 by adding 10 μL 1 M NaOH, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 4 μm LiChroCART Superspher 60 RP Select B (Merck)
Column: 125 × 4 4 μm LiChroCART Superspher 60 RP Select B (Merck)
Mobile phase: MeCN:buffer 30:70 (Buffer was 7.0 g/L K_2HPO_4 in water adjusted to pH 5.8 with 1 M NaOH.)
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 202

CHROMATOGRAM

Retention time: 10
Internal standard: lidocaine (5)
Limit of quantitation: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Sattler,A.; Krämer,I.; Jage,J.; Vrana,S.; Kleemann,P.P.; Dick,W. Development of a HPLC-system for quantitative measurement of lidocaine and bupivacaine in patients plasma during postoperative epidural pain therapy, *Pharmazie*, **1995**, *50*, 741–744.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 264

CHROMATOGRAM

Retention time: 5.43

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; op-

ipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 4 mL MeOH and 4 mL water. 1 mL Serum or 300-500 μL ultrafiltrate + lidocaine, add to the SPE cartridge, wash with 5 mL water, wash with 2 mL MeOH:water 5:95, wash with 2 mL EtOH:water 2.5:97.5, wash with 2 mL MeCN:water 10:90, elute with 1 mL MeCN:50 mM pH 2.4 phosphate buffer 25:75, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 4.6 μBondapak
Mobile phase: MeCN:50 mM pH 4.0 KH_2PO_4 25:75
Flow rate: 1.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Internal standard: lidocaine
Limit of detection: 10-15 ng/mL

KEY WORDS

serum; ultrafiltrate; SPE

REFERENCE

Mazoit,J.X.; Cao,L.S.; Samii,K. Binding of bupivacaine to human serum proteins, isolated albumin and isolated α-1-acid glycopotein. Differences between the two enantiomers are partly due to cooperativity, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 109–115.

SAMPLE

Matrix: blood

Sample preparation: Filter (0.22 μm) plasma, add ropivacaine (590 ng per 1 mL plasma), inject a 500 μL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 7 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 C18-alkyl-diol silica (J.Chromatogr.B 1995, 666, 315; J.Pharm.Biomed.Anal. 1995, 13, 615); B 10 × 4.6 5 μm Kromasil C8 (Eka Nobel) + 100 × 4.6 5 μm Kromasil C18 (Eka Nobel)
Mobile phase: A Isopropanol:buffer 5:95; B MeOH:buffer 63:37 (Prepare buffer by mixing 36.68 mL 1 M phosphoric acid with water, adjust pH to 7.4 with 1 M NaOH, make up to 2 L with water.)
Column temperature: 30 (column B)
Flow rate: A 1.5; B 1
Injection volume: 500

Detector: UV 210

CHROMATOGRAM
Retention time: 15
Internal standard: ropivacaine (9)
Limit of detection: 10 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Yu,Z.; Westerlund,D. Direct injection of large volumes of plasma in a column-switching system for the analysis of local anaesthetics. II. Determination of bupivacaine in human plasma with an alkyl-diol silica precolumn, *J.Chromatogr.A*, **1996**, *725*, 149–155.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm) plasma, inject a 500 μL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 10 5 μm semi-permeable surface C8 (Regis); B 10 × 4.6 5 μm Kromasil C8 (Eka Nobel) + 100 × 4.6 5 μm Kromasil C18 (Eka Nobel)
Mobile phase: A Isopropanol:buffer 5:95; B MeOH:buffer 63:37 (Prepare buffer by mixing 36.68 mL 1 M phosphoric acid with 68.34 mL 1 M NaOH and diluting to 2 L with water, pH 7.7.)
Flow rate: A 1.5; B 1
Injection volume: 500
Detector: UV 240

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Extracted: ropivacaine

KEY WORDS
plasma; column-switching

REFERENCE
Yu,Z.; Westerlund,D. Direct injection of large volumes of plasma in a column-switching system for the analysis of local anaesthetics. I. Optimization of semi-permeable surface precolumns in the system and characterization of some interference peaks, *J.Chromatogr.A*, **1996**, *725*, 137–147.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 200 μL 2 μg/mL etidocaine in water + 1 mL 100 mM pH 9.0 sodium tetraborate + 5 mL diethyl ether, shake for 20 min, centrifuge at 1200 g. Remove the organic layer and add it to 500 μL 200 mM HCl, shake for 20 min, centrifuge at 1200 g. Remove the aqueous layer and add it to 1 mL 100 mM pH 9.0 sodium tetraborate, add 5 mL diethyl ether, shake for 20 min, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness under a stream of air at 50°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nucleosil C8

Mobile phase: THF:10 mM pH 2.4 potassium phosphate 8:92
Flow rate: 1.6
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 4.15
Internal standard: etidocaine (10.60)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics

REFERENCE
Lindberg,R.L.P.; Kanto,J.H.; Pihlajamäki,K.K. Simultaneous determination of bupivacaine and its two metabolites, desbutyl- and 4'-hydroxybupivacaine, in human serum and urine, *J.Chromatogr.*, **1986**, *383*, 357–364.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 μL 5 μg/mL IS in water + 100 μL 1 M pH 10 sodium carbonate buffer, shake by hand for 5 s, add 6 mL n-hexane:isopropanol 5:1, rotate on a tumble-mixer at 28 rpm for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 120 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:20 mM pH 6 sodium phosphate buffer 15:40:45
Flow rate: 1.2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 9.2
Internal standard: 1-pentyl-2-(2',6'-xylylcarbamoyl)piperidine hydrochloride (13.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: fentanyl, morphine, oxycodone

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kastrissios,H.; Hung,M.-F.; Triggs,E.J. High-performance liquid chromatographic method for the quantitation of bupivacaine, 2,6-pipecoloxylidide and 4'-hydroxybupivacaine in plasma and urine, *J.Chromatogr.*, **1992**, *577*, 103–107.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 10 μL 100 μg/mL etidocaine, add to a 3 mL Extrelut SPE cartridge, elute with

15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL 10 mM HCl, add 3 mL diethyl ether, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 11
Internal standard: etidocaine (14)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine, prilocaine, dibucaine
Also analyzed: procaine, butacaine, tetracaine, p-aminobenzoic acid, articaine, o-toluidine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 5% bupivacaine hydrochloride injection and 25 mg/mL morphine sulfate injection with 0.9% NaCl injections to a bupivacaine concentration of 625 μg/mL and a morphine concentration of 100 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:100 mM pH 5 phosphate buffer 40:60
Flow rate: 1.5
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: morphine

KEY WORDS
injections; stability indicating

REFERENCE
Johnson,C.E.; Christen,C.; Perez,M.M.; Ma,M. Compatibility of bupivacaine hydrochloride and morphine sulfate, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 61–64.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot

HPLC VARIABLES
Column: 100 × 3.9 4 μm Radial Pak phenyl (Waters)
Mobile phase: MeOH:buffer 65:35 (Buffer was 5 mM pH 4.8 phosphate buffer containing 1.4 mM tetrabutylammonium hydroxide.)
Flow rate: 3
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: degradation products, fentanyl

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Tu,Y.H.; Stiles,M.L.; Allen,L.V.,Jr. Stability of fentanyl citrate and bupivacaine hydrochloride in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1990**, *47*, 2037–2040.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere XL-ODS C18
Mobile phase: MeCN:100 mM pH 5 KH_2PO_4 40:60
Flow rate: 1.5
Detector: UV 254

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Jones,J.W.; Davis,A.T. Stability of bupivacaine hydrochloride in polypropylene syringes, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2364–2365.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:20 mM KH_2PO_4 adjusted to pH 6.0 with 1 M KOH 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 14.1
Limit of detection: 254 ng/mL

OTHER SUBSTANCES
Simultaneous: morphine, hydromorphone

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 100 × 8 4 μm Novapak C18
Mobile phase: MeCN:0.092%phosphoric acid + 0.2% triethylamine 26:74
Flow rate: 2
Detector: UV 214

CHROMATOGRAM
Internal standard: lidocaine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Also analyzed: diphenhydramine, diltiazem, metabolites

KEY WORDS
rat; liver

REFERENCE
Hussain,M.D.; Tam,Y.K.; Gray,M.R.; Coutts,K.T. Kinetic interactions of lidocaine, diphenhydramine, and verapamil with diltiazem: A study using isolated perfused rat liver, *Drug Metab.Dispos.*, **1994**, *22*, 530–536.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlor-

promazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: butacaine, lidocaine, benzocaine, tetracaine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES
Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH:isopropanol 90:10 containing 15 mM N-benzyloxycarbonylglycyl-L-proline and 9 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: k' 3.09 (first enantiomer)

KEY WORDS
chiral; α = 1.08

REFERENCE
Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 10.60 (A), 5.79 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, le-

vorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

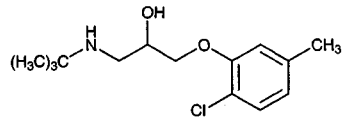
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Bupranolol



Molecular formula: $C_{14}H_{22}ClNO_2$
Molecular weight: 271.79
CAS Registry No.: 14556-46-8, 15148-80-8 (HCl)
Merck Index: 1521

SAMPLE

Matrix: solutions
Sample preparation: Mix 300 µL of a solution in chloroform with 20 µL 0.1% (+)-(S)-naphthylethylisocyanate in chloroform. Mix, let stand at room temperature for 20 min. Evaporate to dryness. Redissolve the residue in 300 µL mobile phase. Inject a 50 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 5 silica (Phenomenex)
Mobile phase: Hexane:chloroform:MeOH 74:25:1
Flow rate: 1
Injection volume: 50
Detector: UV 282

CHROMATOGRAM

Retention time: 8.1, 10.6 (enantiomers)

OTHER SUBSTANCES

Simultaneous: mefloquine

KEY WORDS

chiral; derivatization; siliconize glassware; normal phase; normal phase is superior to reverse-phase procedure

REFERENCE

Souri,E.; Farsam,H.; Jamali,F. Stereospecific determination of mefloquine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 215–222.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 µm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.85

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the mini-

mum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 50:50:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 29.4, 36.5 (enantiomers)
Limit of detection: 0.00125-0.00161 fmole

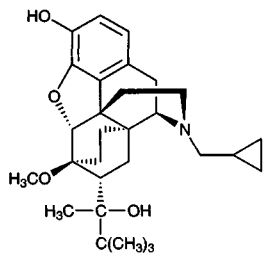
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Buprenorphine



Molecular formula: $C_{29}H_{41}NO_4$
Molecular weight: 467.65
CAS Registry No.: 52485-79-7, 53152-21-9 (HCl)
Merck Index: 1522
Lednicer No.: 2 321

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 µL 500 ng/mL IS in MeOH + 100 µL 1 M NaOH, mix, extract with 6 mL hexane:isopropanol 99:1 by rotary mixing for 10 min, centrifuge at 2000 g for 5 min. Add 300 µL 200 mM HCl to the organic layer, mix, centrifuge at 2000 g for 5 min, discard the upper organic layer, inject a 120 µL aliquot of the acidic aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 Spherisorb C8
Mobile phase: MeCN:triethylamine:Pic B5:60 mM pH 6.4 phosphate buffer 48:0.05:0.15:52, adjusted to pH 6.4 with orthophosphoric acid
Flow rate: 1.6
Injection volume: 120
Detector: UV 214

CHROMATOGRAM

Retention time: 5.2
Internal standard: clothiapine (9.6)
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amitriptyline, amoxapine, carpipramine, clomipramine, demexiptilline, desipramine, diazepam, imipramine, medifoxamine, metapramine, methadone, mianserine, naloxone, normaprotiline, opipramol, paroxetine, quinupramine, tianeptine, trazodone, viloxazine
Noninterfering: codeine, codethyline, morphine, pholcodeine
Interfering: dosulepin, doxepin, maprotiline, nortriptyline, oxaflozane

KEY WORDS

plasma

REFERENCE

Lagrange,F.; Pehourcq,F.; Baumvieille,M.; Begaud,B. Determination of buprenorphine in plasma by liquid chromatography: application to heroin-dependent subjects, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1295–1300.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 5 µg/mL nalbuphine in water + 1 mL 500 mM pH 9.25 sodium carbonate buffer + 3 mL hexane:isoamyl alcohol 9:1, mix on a rotary shaker for 30 min, centrifuge at 1880 g for 20 min, freeze at -20° for 1 h (for rabbit plasma perform on half-scale). Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute in 250 µL mobile phase, inject a 200 µL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Applied Biosystems pre-column
Column: 100 × 2 10 μm μPorasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: F ex 210 em 345

CHROMATOGRAM

Retention time: 8.83
Internal standard: nalbuphine (11.7)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: butorphanol, morphine, ethylmorphine, codeine, nalorphine, fentanyl, meperidine, tramadol
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine

KEY WORDS

plasma; human; pig; dog; rabbit; pharmacokinetics

REFERENCE

Ho,S.-T.; Wang,J.-J.; Ho,W.; Hu,O.Y.-P. Determination of buprenorphine by high-performance liquid chromatography with fluorescence detection: application to human and rabbit pharmacokinetic studies, *J.Chromatogr.*, **1991**, *570*, 339–350.

SAMPLE

Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 26
Limit of detection: 0.30 ppm

OTHER SUBSTANCES

Extracted: caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS

whole blood

REFERENCE

Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 288

CHROMATOGRAM

Retention time: 5.36

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen;

tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, hair, urine

Sample preparation: Blood, urine. Mix 2 mL whole blood, plasma, or urine with 10 ng IS, 1.5 mL saturated pH 8.4 ammonium hydrogen phosphate buffer, and 5 mL chloroform: isopropanol:n-heptane 25:10:65. (Caution! Chloroform is a carcinogen!) Agitate, centrifuge at 3500 g for 10 min, evaporate the organic layer at 45° for 30 min (Speed Vac Concentrator). Reconstitute the dry extract in 20 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 2 μL aliquot of the supernatant. Hair. Decontaminate hair with two portions of dichloromethane for 2 min, pulverize for 5 to 10 min (Retsch MM2 ball mill). Mix 40 mg powdered hair with 1 ng IS and incubate in 1 mL 100 mM HCl overnight at 56°. Neutralize 1 mL hair homogenate using 1 mL 100 mM NaOH, add 1.5 mL saturated pH 8.4 ammonium hydrogen phosphate, extract with 5 mL chloroform:isopropanol:n-heptane 25:10:65. Agitate mixture, centrifuge at 3500 g for 10 min, evaporate the organic layer at 45° for 30 min (Speed Vac Concentrator). Reconstitute the dry extract in 20 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 2 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 1.0 5 μm Opti-Guard C18 (Interchim)
Column: 150 × 2.0 4 μm NovaPak C18 (Waters)
Mobile phase: MeCN:2 mM pH 3.0 ammonium acetate buffer 80:20
Flow rate: 0.2
Injection volume: 2
Detector: MS, Perkin Elmer Sciex API-100, ionspray interface, a post column split 1:3 reduces the flow rate to 50 μL/min for infusion into HPLC/MS interface, nebulizing gas nitrogen (99.95%, 40 psi, FR 1.16 L/min) and curtain gas (FR 1.08 L/min). Ion sampling at +50 V, electron multiplier +2400 V, mass range m/z 260-475, MIM at m/z 414 (norbuprenorphine), 468 (buprenorphine), and 472 (buprenorphine-d4)

CHROMATOGRAM

Retention time: 5.84
Internal standard: buprenorphine-d4 (Radian) (5.79)
Limit of detection: 100 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; whole blood

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. HPLC/MS determination of buprenorphine and norbuprenorphine in biological fluids and hair samples, *J.Forensic Sci.*, **1997**, *42*, 111–114.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 1 mL 40 μm Supelclean SPE cartridge with 4 mL MeOH and 4 mL water. Add 5 mL serum, plasma, or urine to the SPE cartridge, add 400 μL 50 mM borate buffer adjusted to pH 9.1 with 1 M NaOH, wash with 600 μL MeCN:50 mM NaH_2PO_4 20:80, elute slowly with 3 mL chloroform. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 50 mM NaH_2PO_4 in MeCN:water 50:50 (pH adjusted to 2.5 with phosphoric acid) by shaking at 70° for 20 min, dilute with 3 mL water, add 500 μL 50 mM borate buffer adjusted to pH 9.1 with 1 M NaOH, inject a 2 mL sample onto column A with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B for 8 min and monitor the effluent. At the end of the process backflush column B with mobile phase B for 8 min.

HPLC VARIABLES

Column: A 4 × 4 5 μm RP-18 (E. Merck); B 30 × 4 5 μm C18 (Macherey & Nagel)
Mobile phase: A MeCN:50 mM phosphate buffer adjusted to pH 8.5 with 1 M NaOH 20:80 (Use a 250 × 4 column of 40 μm silica between pump and injector to protect column A.); B MeCN:50 mM NaH_2PO_4 25:75
Flow rate: A 2; B 0.8
Injection volume: 2000
Detector: E, ESA Coulochem 5100 A detector, Model 5020 guard cell 500 mV, Model 5010 analytical cell, detector 1 160 mV, detector 2 480 mV, monitor detector 2

CHROMATOGRAM

Retention time: 5
Limit of detection: 0.04 ng/mL

KEY WORDS

serum; plasma; SPE; column-switching; pharmacokinetics

REFERENCE

Schleyer,E.; Lohmann,R.; Rolf,C.; Gralow,A.; Kaufmann,C.C.; Unterhalt,M.; Hiddemann,W. Column-switching solid-phase trace-enrichment high-performance liquid chromatographic method for measurement of buprenorphine and norbuprenorphine in human plasma and urine by electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 275–283.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 212.2

CHROMATOGRAM
Retention time: 14.035

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 mm long C18
Mobile phase: MeCN:5 mM heptanesulfonic acid adjusted to pH 3.5 with glacial acetic acid 35:65
Injection volume: 20
Detector: UV 212

CHROMATOGRAM
Limit of quantitation: 80 ng/mL

KEY WORDS
nasal solutions

REFERENCE
Gries,W.J.; Wan,W.; Matos,F.J.; de Meireles,J.C.; Pimplaskar,H.K.; Sileno,A.P.; Romeo,V.D.; Xia,W.J.; Behl,C.R. A specific and sensitive method for quantitating buprenorphine hydrochloride in a nasal solution (Abstract 2517), *Pharm.Res.*, **1997**, *14*, S381–S381.

SAMPLE
Matrix: hair
Sample preparation: Wash 50 mg hair twice with 5 mL dichloromethane for 2 min, pulverize in a ball mill, incubate in 1 mL 100 mM HCl overnight at 56°, neutralize with 1 mL 100 mM NaOH, add 1 mL pH 8.5 phosphate buffer, extract with 5 mL toluene, agitate, centrifuge. Remove the organic phase and extract it with 1 mL 100 mM HCl. Remove the aqueous phase and add it to 1 mL 100 mM NaOH, 1 mL pH 8.5 phosphate buffer, ann 5 mL toluene. Agitate, centrifuge, remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb CN
Mobile phase: MeCN:10 mM pH 4.0 phosphate buffer:1-heptanesulfonic acid:butylamine 17:85:2:0.01
Flow rate: 1
Injection volume: 60
Detector: E, ESA Coulochem II, first electrode +150 mV, second electrode +500 mV

CHROMATOGRAM
Retention time: 10.71
Limit of detection: 20 ng/g

OTHER SUBSTANCES
Extracted: norbuprenorphine

REFERENCE

Kintz,P. Determination of buprenorphine and its dealkylated metabolite in human hair, *J.Anal.Toxicol.*, **1993**, *17*, 443–444.

SAMPLE

Matrix: hair

Sample preparation: Wash 50 mg hair with 5 mL dichloromethane for 2 min, repeat wash, pulverize in a ball mill, add 1 mL 100 mM HCl, heat at 56° overnight, neutralize, add 1 mL pH 8.5 saturated phosphate buffer, add 10 mL toluene, agitate, centrifuge. Remove the organic layer and add it to 5 mL 100 mM HCl, extract. Remove the aqueous layer and add it to 1 mL ammonia solution, add 1 mL ph 8.5 phosphate buffer, add 5 mL toluene, agitate, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrosorb CN
Mobile phase: MeCN:10 mM pH 4.0 phosphate buffer:1-heptanesulfonic acid 85:17:0.01 (?)
Flow rate: 1
Injection volume: 60
Detector: E, ESA Coulochem II, first electrode +0.15 V, second electrode +0.50 V (monitored)

CHROMATOGRAM

Limit of detection: 0.02 ng/g

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Kintz,P.; Cirimele,V.; Edel,Y.; Jamey,C.; Mangin,P. Hair analysis for buprenorphine and its dealkylated metabolite by RIA and confirmation by LC/ECD, *J.Forensic Sci.*, **1994**, *39*, 1497–1503.

SAMPLE

Matrix: perfusate

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:pH 5.0 buffer 45:55
Flow rate: 1
Detector: UV 210

REFERENCE

Roy,S.D.; Roos,E.; Sharma,K. Transdermal delivery of buprenorphine through cadaver skin, *J.Pharm.Sci.*, **1994**, *83*, 126–130.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: C8 Brownlee OSS Spheri-5
Column: 220 × 4.6 C8 Brownlee OSS Spheri-5
Mobile phase: MeCN:MeOH:10 mM pH 5 phosphate buffer 59.5:25.5:15
Flow rate: 1.5
Injection volume: 500
Detector: UV 215

OTHER SUBSTANCES
Also analyzed: prodrugs

REFERENCE
Stinchcomb,A.L.; Paliwal,A.; Dua,R.; Imoto,H.; Woodard,R.W.; Flynn,G.L. Permeation of buprenorphine and its 3-alkyl-ester prodrugs through human skin, *Pharm.Res.*, **1996**, *13*, 1519–1523.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.39

OTHER SUBSTANCES
Simultaneous: tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 OSS Spheri-5 C8
Mobile phase: MeCN:MeOH:pH 5 acetate buffer 25.5:59.5:15
Flow rate: 1.5
Detector: UV 285

OTHER SUBSTANCES
Simultaneous: prodrugs

REFERENCE
Stinchomb,A.L.; Dua,R.; Paliwal,A.; Woodard,R.W.; Flynn,G.L. A solubility and related physicochemical property comparison of buprenorphine and its 3-alkyl esters, *Pharm.Res.*, **1995**, *12*, 1526–1529.

SAMPLE
Matrix: tissue
Sample preparation: Extract skin with 10 mL MeCN by gentle agitation overnight. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Sheri-5 C8 (Brownlee)
Mobile phase: MeCN:MeOH:pH 5.0 phosphate buffer 60:25:15
Flow rate: 1.5
Injection volume: 20
Detector: UV 215

OTHER SUBSTANCES
Extracted: acetylbuprenorphine, butylbuprenorphine, isobutylbuprenorphine

KEY WORDS
mouse; skin

REFERENCE
Imoto,H.; Zhou,Z.; Stinchcomb,A.L.; Flynn,G.L. Transdermal prodrug concepts: Permeation of buprenorphine and its alkyl esters through hairless mouse skin and influence of vehicles, *Biol.Pharm.Bull.*, **1996**, *19*, 263–267.

SAMPLE
Matrix: urine
Sample preparation: Buffer the urine sample at pH 9.7. Extract with ethyl acetate:heptane (4:1). Dry the organic phase and reconstitute with mobile phase.

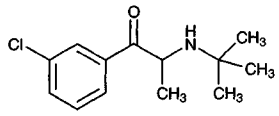
HPLC VARIABLES
Column: Hypersil silica
Mobile phase: MeCN:0.06% trifluoroacetic acid 90:10
Detector: MS, Sciex API III, heated nebulizer, positive ion mode

CHROMATOGRAM
Limit of quantitation: 0.2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, naloxone, norbuprenorphine

REFERENCE
Johnson,R.A.; Haan,D.E.; James,C.A.; Hopkins,N.K. Determination of linezolid, PNU-100766, in human plasma and urine using high-performance liquid chromatography with ultraviolet detection (Abstract 2487), *Pharm.Res.*, **1997**, *14*, S374–S374.

Bupropion



Molecular formula: $C_{13}H_{18}ClNO$
Molecular weight: 239.76
CAS Registry No.: 34911-55-2, 31677-93-7 (HCl)
Merck Index: 1523
Lednicer No.: 2 124

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 150 ng IS + 1 mL 600 mM pH 9.5 carbonate buffer + 10 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix for 10 min, centrifuge at 1500 g for 10 min, inject an aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-1 (Supelco)
Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM KH_2PO_4 containing 7 mM sodium heptanesulfonate and 10 mM triethylamine, adjust pH to 3.0 with phosphoric acid.)
Column temperature: 30
Flow rate: 2.3
Detector: UV 254 (UV 214 for metabolites)

CHROMATOGRAM

Retention time: 11.5
Internal standard: 2-(tert-butylamino)-4'-fluorovalerophenone hydrochloride (15.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: amitriptyline, amoxapine, chlorimipramine, chlorpromazine, desipramine, desmethyldoxepin, doxepin, fluphenazine, haloperidol, imipramine, loxapine, maprotiline, mianserin, nortriptyline, perphenazine, thioridazine, trazodone

KEY WORDS

plasma

REFERENCE

Cooper,T.B.; Suckow,R.F.; Glassman,A. Determination of bupropion and its major basic metabolites in plasma by liquid chromatography with dual-wavelength ultraviolet detection, *J.Pharm.Sci.*, **1984**, *73*, 1104–1107.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma or serum + 20 μL 1 μg/mL IS in 100 mM HCl + 400 μL 100 mM KOH, vortex for 5 s, add 5 mL hexane:isoamyl alcohol 96:4, vortex for 20 s, centrifuge at 2000 rpm for 6 min. Remove the organic layer and add it to 100 μL MeOH: 4 M HCl 99:1, evaporate to dryness under a stream of air at 30-35°, reconstitute the residue in 200 μL MeCN, inject a 60 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Econosphere silica
Mobile phase: MeOH:buffer 95:5 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 3.2 with 50 mM phosphoric acid.)

Flow rate: 0.9
Injection volume: 60
Detector: UV 248

CHROMATOGRAM

Retention time: 4.29
Internal standard: 2-(tert-butylamino)-4'-fluorobutyrophenone (234U66) (4.04)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amitriptyline, amoxapine, benzphetamine, chlorpromazine, clozapine, desipramine, diethylpropion, diphenhydramine, haloperidol, imipramine, loxapine, nortriptyline, promethazine, propiomazine, sertraline, thioridazine, trazodone, trifluprom azine, trimeprazine
Noninterfering: chlorpheniramine, mesoridazine, thiothixene, trifluoperazine, trihexyphenidyl
Interfering: fluoxetine

KEY WORDS

plasma; serum; normal phase

REFERENCE

Jennison,T.A.; Brown,P.; Crossett,J.; Urry,F.M. A high-performance liquid chromatographic method for quantitating bupropion in human plasma or serum, *J.Anal.Toxicol.*, **1995**, *19*, 69–72.

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 500 μL saturated sodium borate, vortex briefly, add 5 mL MTBE, vortex briefly then mix on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min. Remove the organic phase and add it to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer. Tissue. Weigh whole brain and homogenize with 10 mL 340 mM perchloric acid containing 0.01 mM EDTA for 20 s (Brinkman PT 10/35). Remove a 1 mL aliquot and add 500 μL 600 mM sodium carbonate and 3 mL hexane:isoamyl alcohol 98:2 to it. Shake on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min, remove the organic layer and repeat the extraction. Combine the organic layers and add them to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm IBM reverse phase (trimethyl silane)
Mobile phase: MeCN:pH 3.0 phosphate buffer 27:73 containing 20 mM heptanesulfonic acid and 40 mM triethylamine
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 10.6
Internal standard: bupropion

OTHER SUBSTANCES

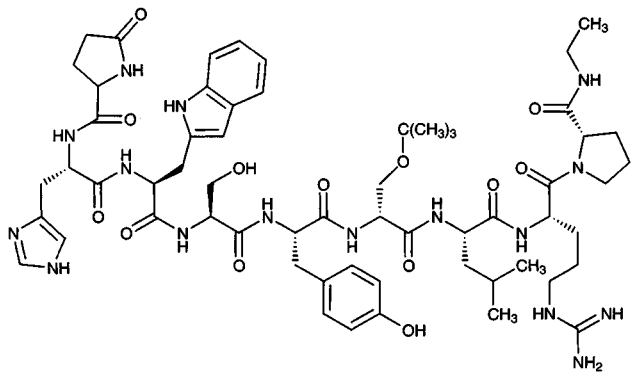
Simultaneous: trazodone

KEY WORDS
plasma; rat; brain; bupropion is IS

REFERENCE
Miller,R.L.; DeVane,C.L. Analysis of trazodone and m-chlorophenylpiperazine in plasma and brain tissue by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 388–393.

Buserelin

Molecular formula: $C_{60}H_{86}N_{16}O_3$
Molecular weight: 1239.44
CAS Registry No.: 57982-77-1, 68630-75-1 (acetate)
Merck Index: 1527



SAMPLE
Matrix: blood, urine
Sample preparation: Extract using a Sep-Pak C18 SPE cartridge.

HPLC VARIABLES
Column: 100 × 8 10 μm Bondapak Rad-Pak
Mobile phase: MeCN:120 mM pH 6.2 KH_2PO_4 65:35
Detector: radioimmunoassay

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Kiesel,L.; Sandow,J.; Bertges,K.; Jerabek-Sandow,G.; Trabant,H.; Runnebaum,B. Serum concentration and urinary excretion of the luteinizing hormone-releasing hormone agonist buserelin in patients with endometriosis, *J.Clin.Endocrinol.Metab.*, **1989**, *68*, 1167–1173.

SAMPLE
Matrix: cell incubations
Sample preparation: Inject a 60 μL aliquot.

HPLC VARIABLES
Guard column: 11 × 4 5 μm Nucleosil C18
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: Gradient. A was MeCN:water 0.5:99.5 containing 0.05% trifluoroacetic acid. B was MeCN:water 35:65 containing 0.05% trifluoroacetic acid. A:B 100:0 for 10 min, to 83:17 (step gradient), to 0:100 over 25 min.
Flow rate: 1
Injection volume: 60
Detector: UV 215 or F ex 280 em 365

CHROMATOGRAM
Retention time: 39

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rat; kidney

REFERENCE
Kertscher,U.; Brudel,M.; Mehlis,B.; Sandow,J.; Berger,H. Pathways of degradation of buserelin by rat kidney membrane, *J.Pharmacol.Exp.Ther.*, **1995**, *273*, 709–715.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 100 μL enzyme incubation (rat nasal mucosa homogenate) to 1 mL 100 mM HCl at 0°.

HPLC VARIABLES
Column: 150 × 6 Inertsil ODS-2
Mobile phase: MeCN:0.8% pH 6.2 KH_2PO_4 1:2
Flow rate: 1
Detector: F ex 280 em 350

KEY WORDS
rat

REFERENCE
Abe,K.; Irie,T.; Uekama,K. Enhanced nasal delivery of luteinizing hormone releasing hormone agonist buserelin by oleic acid solubilized and stabilized in hydroxypropyl-β-cyclodextrin, *Chem.Pharm.Bull.*, **1995**, *43*, 2232–2237.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 23:77
Flow rate: 1
Detector: UV 214

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 3 Spherisorb S5ODS-2
Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$. B was MeCN. A:B from 82:18 to 64:36 over 25 min, maintain at 64:36 for 2.5 min, return to initial conditions over 1 min, re-equilibrate for 6.5 min. or Isocratic MeCN:0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$ 24:76
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 22.5 (gradient), 20 (isocratic)

OTHER SUBSTANCES
Simultaneous: deslorelin, gonadorelin, goserelin, leuprolide, nafarelin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Corran,P.H.; Sutcliffe,N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography, *J.Chromatogr.*, **1993**, *636*, 87–94.

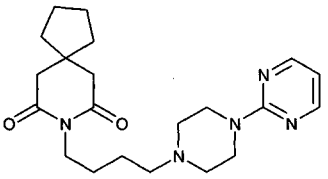
SAMPLE
Matrix: solutions
Sample preparation: 100 μL Incubation solution + 1 mL 100 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 100-5C18
Mobile phase: MeCN:100 mM phosphoric acid 2:7, adjusted to pH 2.5 with triethylamine

KEY WORDS
for buserelin acetate

REFERENCE
Matsubara,K.; Abe,K.; Irie,T.; Uekama,K. Improvement of nasal bioavailability of luteinizing hormone-releasing hormone agonist, buserelin, by cyclodextrin derivatives in rats, *J.Pharm.Sci.*, **1995**, *84*, 1295–1300.

Buspirone



Molecular formula: $C_{21}H_{31}N_5O_2$
Molecular weight: 385.51
CAS Registry No.: 36505-84-7, 33386-08-2 (HCl)
Merck Index: 1528
Lednicer No.: 2 300; 4 119

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 50 mM pH 7.2 KH_2PO_4 buffer. Centrifuge whole blood at 1500 g for 10 min. Add 50 µL 500 ng/mL prazosin to 1 mL plasma, vortex for 5 s, add 1 mL 50 mM pH 7.2 KH_2PO_4 buffer, vortex for 5 s, add to the SPE cartridge, dry in a stream of air, wash with two 1 mL portions of 50 mM pH 7.2 KH_2PO_4 buffer, wash with 500 µL MeOH. Dry the cartridge in a stream of air, let stand for 15 min, elute with 1 mL MeCN:25% ammonium hydroxide 99:1, evaporate the eluate to dryness under a stream of nitrogen, dissolve the residue in 200 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Supelguard ABZ+plus C18 (Supelco)
Column: 250 × 4.6 Supelcosil ABZ+plus C18 (Supelco)
Mobile phase: MeCN:50 mM pH 6.5 KH_2PO_4 buffer 30:70
Flow rate: 1
Detector: E, ESA Coulochem II, 5011 model analytical cell, guard cell +950 mV, first electrode +600 mV, second electrode +900 mV

CHROMATOGRAM

Retention time: 11.24
Internal standard: prazosin (6.14)
Limit of detection: 60 pg/mL
Limit of quantitation: 100 pg/mL

KEY WORDS

plasma; SPE

REFERENCE

Ary,K.; Róna,K.; Ondi,S.; Gachályi,B. High-performance liquid chromatographic method with coulometric detection for the determination of buspirone in human plasma by means of a column-switching technique, *J.Chromatogr.A*, **1998**, *797*, 221–226.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 12.523

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: dialysate
Sample preparation: Inject an aliquot directly.

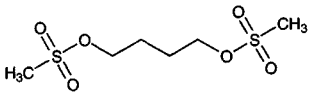
HPLC VARIABLES
Column: 150 × 1 5 μm Sepstik CN-5μ (Bioanalytical Systems)
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 15:85:0.1, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 0.06
Injection volume: 10
Detector: E, BAS 4C, glassy carbon working electrode +1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 1 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
microbore; rat; brain; pharmacokinetics

REFERENCE
Tsai,T.H.; Chen,C.F. Measurement and pharmacokinetic analysis of buspirone by means of brain microdialysis coupled to high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *762*, 269–273.

Busulfan



Molecular formula: $C_6H_{14}O_6S_2$
Molecular weight: 246.31
CAS Registry No.: 55-98-1
Merck Index: 1529

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 20 µL 40 µg/mL 1,6-bis(methanesulfonyloxy)hexane in ethyl acetate + 125 µL 82 mg/mL diethylditiocarbamate (DDTC) in water. Vortex. Add 200 µL MeOH and 2 ml ethyl acetate. Vortex for 10 s and mix by rotation for 5 min. Centrifuge sample at 600 g for 10 min. Transfer organic phase to a glass tube, dry under nitrogen. Reconstitute extract with 100 µL MeOH, vortex, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: Nova-Pak ODS
Column: 150 × 3.9 Nova-Pak ODS
Mobile phase: MeOH:water 80:20
Flow rate: 0.8
Injection volume: 30
Detector: UV 251; MS, electrospray, source temperature 120°, cone voltage 22-26 V

CHROMATOGRAM

Retention time: 8.5
Internal standard: 1,6-bis(methanesulfonyloxy)hexane (16)
Limit of detection: 20 ng/mL (S/N 6)
Limit of quantitation: 60 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, allopurinol, carboplatin, cyclosporine, diazepam, heparin, lorazepam, methotrexate, methylprednisolone, ondansetron, pentoxifylline, phenytoin, prochlorperazine

KEY WORDS

derivatization; validation; plasma

REFERENCE

Heggie,J.R.; Wu,M.; Burns,R.B.; Ng,C.S.; Fung,H.C.; Knight,G.; Barnett,M.J.; Spinelli,J.J.; Embree,L. Validation of a high-performance liquid chromatographic assay method for pharmacokinetic evaluation of busulfan, *J.Chromatogr.B*, **1997**, *692*, 437–444.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Sep-Pak SPE cartridge with seven 1 mL portions of MeOH and two 1 mL portions of water. Add 20 µL 40 µg/mL CGA-112913 solution to 200 µL plasma, vortex, add 200 µL MeCN, vortex for 30 s, centrifuge at 16750 g for 3 min. Add 400 µL water to a 360 µL aliquot of the supernatant then add 200 µL 1.17 M diethyldithiocarbamate in water, vortex for 30 s, rotate for 5 min, add 2 mL ethyl acetate, vortex for 1 min, centrifuge at 5125 g for 10 min. Evaporate a 1.8 mL aliquot of the ethyl acetate layer to dryness under a stream of air at 45°, reconstitute the residue in 200 µL MeOH. Add 500 µL water then the residue dissolved in MeOH to the SPE cartridge and allow them to pass through, wash with two 1 mL portions of MeOH:water 50:50, elute with two 250 µL portions of MeOH and two 500 µL portions of ethyl acetate, combine the eluates, evaporate to dryness under a stream of air at 45°, reconstitute the residue with 200 µL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb-MV (Rainin)
Mobile phase: MeCN:THF:water 55:20:25 (pH 4.2 without modification)
Flow rate: 1.2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: CGA-112913 N-(2,6-difluorobenzoyl)-N-(3,5-dichloro-4-(3-chloro-5-trifluoromethylpyridin-2-yloxy)phenyl)urea
Limit of detection: 150 ng/mL

KEY WORDS
pharmacokinetics; plasma; derivatization; rat; SPE

REFERENCE
Chow,D.S.-L.; Bhagwatwar,H.P.; Phadungpojna,S.; Andersson,B.S. Stability-indicating high-performance liquid chromatographic assay of busulfan in aqueous and plasma samples, *J.Chromatogr.B*, **1997**, *704*, 277–288.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 600 μL MeOH, mix, let stand at -20° for 20 min, centrifuge at 1500 g for 10 min. Add 600 μL supernatant to 150 μL 5% diethyldithiocarbamate and 600 μL 100 mM pH 5.5 ammonium acetate, mix, extract with 1.5 mL ethyl acetate, centrifuge for 1.5 min. Lyophilize a 1 mL aliquot of the extract, reconstitute with 200 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 1
Detector: UV 251

CHROMATOGRAM
Retention time: 12.0
Limit of detection: 200 nM

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Henner,W.D.; Furlong,E.A.; Flaherty,M.D.; Shea,T.C.; Peters,W.P. Measurement of busulfan in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *416*, 426–432.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 1 mL MeOH and 3 mL water. Add 1 mL plasma to the cartridge, wash with 2 mL water, dry, elute with 1 mL MeOH by centrifuging. Remove the eluate and add it to 1 mL 4 M NaI in water and 400 μL n-heptane, heat the mixture in a closed vial with stirring at 70° for 40 min, cool to room temperature. Remove 350 μL of the upper layer and centrifuge it at 12000 g. Remove 250 μL of the upper organic layer and add it to 100 μL 2-methoxyethanol, evaporate the n-heptane without heating under vacuum for 8 min, inject a 20 μL aliquot of the residue.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm LiChrosorb CN

Column: 250 × 4.6 5 μm LiChrosorb CN
Mobile phase: MeOH:water 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 226 following photolysis with a GTE G8T5 germicidal lamp using a 0.8 mm i.d. PTFE knitted tube reactor, internal volume 2.4 mL (or a 25 m × 0.3 mm i.d. tube in a commercial Beam Booster reactor)

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 20 ng/mL

KEY WORDS
plasma; post-column photolysis; derivatization; SPE; pharmacokinetics

REFERENCE
Blanz,J.; Rosenfeld,C.; Proksch,B.; Ehninger,G.; Zeller,K.-P. Quantitation of busulfan in plasma by high-performance liquid chromatography using postcolumn photolysis, *J.Chromatogr.*, **1990**, *532*, 429–437.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bakerbond C18 SPE cartridge with three 1 mL portions of MeOH and two 1 mL portions of water, do not allow to dry. 300 μL Plasma + 30 μL 25 μg/mL IS in MeOH + 150 μL reagent solution, vortex for 10 s, add 2 mL ethyl acetate, vortex briefly, rock for 10 min on a blood mixer, centrifuge at 1500 g for 10 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of air at 70°, reconstitute the residue in 500 μL MeOH, add 500 μL water, add to SPE cartridge, wash with two 1 mL portions of MeOH:water 50:50, elute with two 250 μL portions of MeOH, inject a 20 μL aliquot. (The reagent solution was 8.2 g sodium diethyldithiocarbamate in 100 mL water.)

HPLC VARIABLES
Column: 150 × 4 3 μm MicroPak-SP-C18
Mobile phase: MeCN:water:THF 55:25:20
Flow rate: 0.8
Injection volume: 20
Detector: UV 278

CHROMATOGRAM
Retention time: 4.5
Internal standard: N-(2,6-difluorobenzoyl)-N'-[3,5-dichloro-4-(3-chloro-5-trifluoromethylpyridin-2-yloxy)phenyl]urea (CGA-112913) (5.5)
Limit of detection: 0.4 ng/mL

KEY WORDS
plasma; SPE; derivatization

REFERENCE
MacKichan,J.J.; Bechtel,T.P. Quantitation of busulfan in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *532*, 424–428.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 100 μL water + 5 mL diethyl ether:dichloromethane 70:30, shake for 5 min, centrifuge at 1500 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, recon-

stitute the residue in 250 μL water, inject a 100 μL aliquot onto column A with mobile phase A (t = 0) for derivatization, after 5 min (t = 5) backflush the (derivatized) contents of column A onto column B with mobile phase B, after 3 min (t = 8) remove column A from the circuit and continue to elute column B with mobile phase B. After another 2 min (t = 10) direct the effluent from column B onto column C for 0.6 min (t = 10.6) then elute column C with mobile phase C, monitor the effluent from column C.

HPLC VARIABLES

Column: A 10 × 4 5 μm Inertsil ODS-80A; B 150 × 4.6 5 μm Inertsil ODS-2; C 150 × 6 5 μm Capcell Pak C18 (Shiseido)

Mobile phase: A 1% sodium diethyldithiocarbamate (Use a 10 × 4 5 μm Inertsil ODS-80A column between pump and injector to protect column A, replace every day. Stop flow of mobile phase A from t = 5.1 to t = 32.); B MeCN:20 mM pH 4.6 KH_2PO_4 60:40; B MeCN: 20 mM pH 4.6 KH_2PO_4 65:35

Flow rate: A 0.5; B 1; C 1

Injection volume: 100

Detector: UV 278

CHROMATOGRAM

Retention time: 16

Limit of quantitation: 10 ng/mL

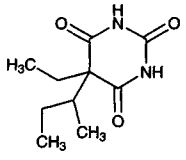
KEY WORDS

serum; column-switching; derivatization; heart-cut

REFERENCE

Funakoshi,K.-i.; Yamashita,K.; Chao,W.-f.; Yamaguchi,M.; Yashiki,T. High-performance liquid chromatographic determination of busulfan in human serum with on-line derivatization, column switching and ultraviolet absorbance detection, *J.Chromatogr.B*, **1994**, *660*, 200–204.

Butabarbital



Molecular formula: $C_{10}H_{15}N_2NaO_3$
Molecular weight: 234.23
CAS Registry No.: 143-81-7, 125-40-6 (free acid)
Merck Index: 1530
Lednicer No.: 1 268

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 15.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE

Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8

Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 4.18
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin
Interfering: chloramphenicol

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, mephobarbital, methabarbital, methohexital, phenobarbital, phenytoin, secobarbital, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 15.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 1.66

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, amobarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30

Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 2 μBondapak C18
Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid
Flow rate: 0.27
Injection volume: 5
Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Simultaneous: butethal, butalbital, talbutal, amobarbital, pentobarbital

KEY WORDS
mass spectra given

REFERENCE
Ryan,T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J.Liq.Chromatogr.*, **1994**, *17*, 867–881.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.60 (A), 4.92 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen,

nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.85

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8

Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min
Flow rate: 1
Injection volume: 20
Detector: UV 212

CHROMATOGRAM

Retention time: 8.7
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: barbital, allobarbital, phenobarbital, pentobarbital, secobarbital
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, trifluprom azine, trimethoprim, trimipramine

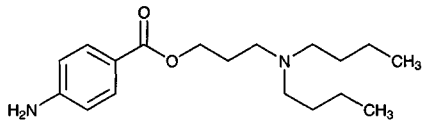
KEY WORDS

SPE

REFERENCE

Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Butacaine



Molecular formula: $C_{18}H_{30}N_2O_2$
Molecular weight: 306.45
CAS Registry No.: 149-16-6, 149-15-5 (sulfate)
Merck Index: 1531
Lednicer No.: 1 12

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-

thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

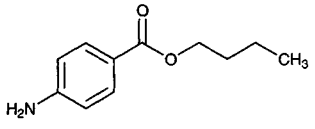
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, per-

santine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Butamben



Molecular formula: $C_{11}H_{15}NO_2$
Molecular weight: 193.25
CAS Registry No.: 94-25-7, 577-49-0 (picrate)
Merck Index: 1538

SAMPLE

Matrix: solutions
Sample preparation: Inject a 5 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 5
Detector: UV 305

CHROMATOGRAM

Retention time: 14

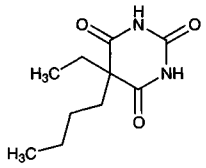
OTHER SUBSTANCES

Simultaneous: benzocaine, lidocaine, pramoxine, procaine, tetracaine

REFERENCE

Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 569–570.

Butethal



Molecular formula: $C_{10}H_{16}N_2O_3$
Molecular weight: 212.25
CAS Registry No.: 77-28-1
Merck Index: 1550
Lednicer No.: 1 268

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.858

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

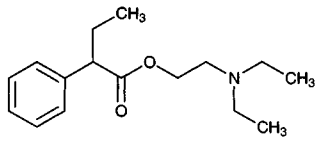
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Butethamate



Molecular formula: $C_{16}H_{25}NO_2$
Molecular weight: 263.38
CAS Registry No.: 14007-64-8, 3639-12-1 (citrate)
Merck Index: 1551

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

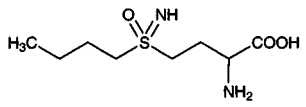
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine,

quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Buthionine sulfoximine



Molecular formula: $C_8H_{18}N_2O_3S$
Molecular weight: 222.31
CAS Registry No.: 5072-26-4
Merck Index: 1556

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 50 μL 100 μg/mL L-norvaline, add 10% sulfosalicylic acid, mix, centrifuge at 13000 rpm for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL 400 mM pH 9.53 phosphate buffer, add 50 μL 3 mg/mL dansyl chloride in acetone, heat at 40° for 10 min, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeCN:MeOH:10 mM pH 2.1 phosphate buffer 3:1:8
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: F ex 335 em 525

CHROMATOGRAM

Retention time: 5
Internal standard: norvaline (10)
Limit of detection: 10 μg/mL

KEY WORDS

derivatization; rat; plasma; protect from light

REFERENCE

Koyama,H.; Sugioka,N.; Hirata,I.; Ohta,T.; Kishimoto,H. Determination of L-buthionin (SR)-sulfoximine, γ-glutamylcysteine synthetase inhibitor in rat plasma with HPLC after prelabeling with dansyl chloride, *J.Chromatogr.Sci.*, **1996**, *34*, 326–329.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL 100 μg/mL L-norleucine in 100 mM HCl, vortex, filter (Millipore 10000 nominal molecular weight limit) while centrifuging at 5000 g for 15 min. Remove a 40 μL aliquot of the ultrafiltrate and add it to 80 μL freshly-prepared EtOH:phenylisothiocyanate:triethylamine 40:1:1, vortex, let stand at room temperature for 15 min, add 10 μL 316 mg/mL L-serine, let stand for 15 min, evaporate to dryness under reduced pressure, reconstitute with 600 μL A, inject a 250 μL aliquot. Urine. Condition a Bond Elut C18 SPE cartridge with 3 mL MeOH and 3 mL water. 100 μL Urine + 100 μL 100 μg/mL L-norleucine in 100 mM HCl, vortex, add to the SPE cartridge, elute with 300 μL 100 mM HCl, elute with 500 μL MeCN:100 mM HCl 30:70, collect all the effluent from the cartridge, vortex thoroughly. Remove a 40 μL aliquot and add it to 80 μL freshly-prepared EtOH:phenylisothiocyanate:triethylamine 40:1:1, vortex, let stand at room temperature for 15 min, add 10 μL 316 mg/mL L-serine, let stand for 15 min, evaporate to dryness under reduced pressure, reconstitute with 600 μL A, inject a 250 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Adsorbosphere c18

Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: Gradient. A:B 91.5:8.5 for 30 min, to 55:45 over 10 min, to 0:100 over 2 min, maintain at 0:100 for 6 min, return to initial conditions over 2 min, re-equilibrate for 8 min. A was MeCN:140 mM pH 6.40 sodium acetate buffer containing 500 μL/L triethylamine and 200 μL/L 1 mg/mL EDTA 6:94. B was MeCN:water 60:40 containing 200 μL/L 1 mg/mL EDTA.
Flow rate: 1.25
Injection volume: 250
Detector: UV 254

CHROMATOGRAM

Retention time: 26.0 (S), 26.6 (R)
Internal standard: L-norleucine (39.3)
Limit of detection: 1 μg/mL (plasma)
Limit of quantitation: 2 μg/mL (plasma), 10 μg/mL (urine), 6 μg/mL (urine)

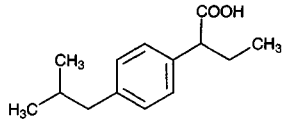
KEY WORDS

derivatization; plasma; ultrafiltrate; SPE

REFERENCE

Brennan,J.M.; O'Dwyer,P.J.; Ozols,R.F.; LaCreta,F.P. High-performance liquid chromatographic determination of the *S*- and *R*-diastereoisomers of L-buthionine (*SR*)-sulfoximine in human plasma and urine, *J.Chromatogr.*, **1993**, *620*, 121–128.

Butibufen



Molecular formula: $C_{14}H_{20}O_2$
Molecular weight: 220.31
CAS Registry No.: 55837-18-8
Merck Index: 1557

SAMPLE

Matrix: bulk, formulations

Sample preparation: Raw materials. Dissolve in MeCN to a concentration of 10 mg/mL, inject a 3 μL aliquot. Microemulsions. Dilute 0.5-2 g microemulsion to 5 mL with MeCN, add this to a previously activated C18 Sep-Pak SPE cartridge, wash four times with MeCN, collect eluate and make up to 10 mL with MeCN, inject a 3 μL aliquot. Cream. 0.5-2 g Cream + 3 mL MeCN, shake vigorously at 55° for 10 min, add 3 mL MeCN, cool, centrifuge at 3500 rpm for 20 min. Remove the supernatant and filter (0.2 μm), repeat extraction three times, make up to 25 mL with MeCN, pass through a previously conditioned Sep-Pak C18 cartridge, inject a 15 μL aliquot. Microencapsulated formulations. Weigh out an amount of microencapsulated formulation corresponding to 50-300 mg butibufen, dilute with 2 mL water, stir at 40° until the coating had dissolved, make up to 10 mL with MeCN, filter (0.45 μm), inject a 3 μL aliquot. Tablets, sachets. Pulverize, weigh out amount containing 250-1500 mg butibufen, add 10 mL water, stir at 40° for 15 min, dilute to 50 mL with MeCN, filter (0.45 μm), inject a 3 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:water:orthophosphoric acid 472:548:0.4
Column temperature: 40
Flow rate: 1
Injection volume: 3-15
Detector: UV 264

CHROMATOGRAM

Retention time: 10.1

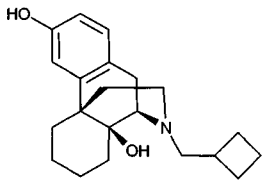
KEY WORDS

SPE; microemulsions; creams; microencapsulated; tablets; sachets

REFERENCE

González Tavares,L.; Pérez de la Cruz,M.J.; Sanz Saiz,P.; Camacho,M.A.; Martin,J.L. High pressure liquid chromatographic determination of the new non-steroidal anti-inflammatory agent butibufen, *Arzneimittelforschung*, **1992**, *42*, 818–820.

Butorphanol



Molecular formula: $C_{21}H_{29}NO_2$
Molecular weight: 327.47
CAS Registry No.: 42408-82-2, 58786-99-5 (tartrate)
Merck Index: 1565
Lednicer No.: 2 325

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 μL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 3:1. Evaporate the eluate to dryness under reduced pressure, dissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Hitachi gel 3056 octadecylsilica
Mobile phase: MeOH:100 mM ammonium acetate 60:40
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M1000, APCI, nebulizer 260°, vaporizer 399

CHROMATOGRAM

Retention time: 3.8
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: atipamezole, atropine, flumazenil, ketamine, medetomidine, midazolam, xylazine

KEY WORDS

plasma; SPE; dog

REFERENCE

Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in mobile phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Applied Biosystems pre-column
Column: 100 × 2 10 μm μPorasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 9.31
Limit of detection: 6.3 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, morphine, ethylmorphine, codeine, nalbuphine, nalorphine, meperidine, tramadol

Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine

Interfering: fentanyl

REFERENCE

Ho,S.-T.; Wang,J.-J.; Ho,W.; Hu,O.Y.-P. Determination of buprenorphine by high-performance liquid chromatography with fluorescence detection: application to human and rabbit pharmacokinetic studies, *J.Chromatogr.*, **1991**, *570*, 339–350.

SAMPLE

Matrix: urine

Sample preparation: Condition a 1 mL Cyano Bond Elut SPE cartridge with 2 mL MeOH then 2 mL 10 mM pH 6.0 ammonium acetate, do not allow to dry. 1 mL Urine + 100 μL 1 M pH 6.0 ammonium acetate + 50 μL 500 ng/mL IS in water, vortex for 20 s, add to SPE cartridge, wash with 2 mL 10 mM pH 6.0 ammonium acetate, wash with 2 mL MeCN, dry cartridge under vacuum for 1 min. Elute with two 1 mL portions of MeCN: triethylamine 99:1. Evaporate eluate to dryness under nitrogen at 30°, reconstitute in 150 μL MeCN:MeOH:water 20:10:70 containing 10 mM ammonium acetate and 10 mM tetramethylammonium hydroxide adjusted to pH 5.0 with glacial acetic acid, vortex for 30 s, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:water:buffer 20:10:69:1 (Buffer was 1 M ammonium acetate and 1 M tetramethylammonium hydroxide adjusted to pH 6.0 with glacial acetic acid.)

Flow rate: 1

Injection volume: 75

Detector: F ex 200 em 325

CHROMATOGRAM

Retention time: 23.4

Internal standard: BC-2605 (cyclopropyl analog of butorphanol) (13.0)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, antipyrine, procainamide, aspirin, lidocaine, furosemide, dextrorphan, dextromethorphan, metabolites

Noninterfering: cimetidine, ibuprofen, N-acetylprocainamide

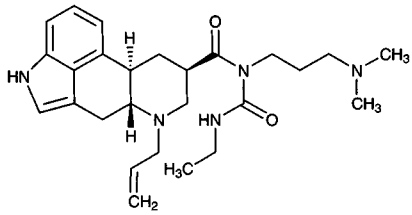
KEY WORDS

SPE

REFERENCE

Willey,T.A.; Duncan,G.F.; Tay,L.K.; Pittman,K.A.; Farmen,R.H. High-performance liquid chromatographic method for the quantitative determination of butorphanol, hydroxybutorphanol, and norbutorphanol in human urine using fluorescence detection, *J.Chromatogr.B*, **1994**, *652*, 171–178.

Cabergoline



Molecular formula: $C_{26}H_{37}N_5O_2$
Molecular weight: 451.61
CAS Registry No.: 81409-90-7, 85329-89-1 (diphosphate)
Merck Index: 1637

SAMPLE

Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 0.5 (plasma) or 1 (urine) mL 500 mM pH 9 borate buffer + 2.5 mL dichloromethane:isooctane 40:60, shake on a rotary mixer for 5 min, centrifuge at 1200 g for 15 min, repeat extraction. Combine the organic layers and add them to 100 μL 100 mM phosphoric acid, vortex for 1 min, centrifuge at 1200 g for 10 min. Remove the aqueous phase and add it to 1 mL n-hexane, vortex, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:75 mM pH 3 phosphate buffer 20:80
Flow rate: 0.6
Injection volume: 50
Detector: E, ESA Model 5100 Coulochem, Model 5020 guard cell +670 mV, Model 5011 analytical cell, detector 1 +350 mV, detector 2 +650 mV

CHROMATOGRAM

Retention time: 13.5
Limit of quantitation: 0.30 ng/mL (urine), 0.25 ng/mL (plasma)

OTHER SUBSTANCES

Noninterfering: levodopa, methyldopa, dopamine, homovanillic acid

KEY WORDS

plasma

REFERENCE

Pianezzola,E.; Bellotti,V.; La Croix,R.; Strolin Benedetti,M. Determination of cabergoline in plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *574*, 170–174.

SAMPLE

Matrix: urine
Sample preparation: Add urine to Amberlite XAD-2 resin (resin/urine = 0.35-0.40), wash with water, elute with acetone, elute with acetic acid. Combine aliquots of the eluates, evaporate to dryness under vacuum, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Guardpak C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:100 mM pH 2 KCl/HCl buffer 12:88 for 5 min, to 30:70 over 15 min, maintain at 30:70 for 15 min
Flow rate: 1
Injection volume: 200
Detector: UV 280

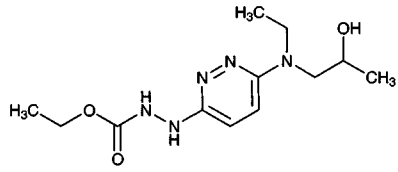
CHROMATOGRAM
Retention time: 25.4

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; monkey; human; SPE

REFERENCE
Battaglia,R.; Strolin Benedetti,M.; Mantegani,S.; Castelli,M.G.; Cocchiara,G.; Dostert,P. Disposition and urinary metabolite pattern of cabergoline, a potent dopaminergic agonist, in rat, monkey and man, *Xenobiotica*, **1993**, *23*, 1377–1389.

Cadralazine



Molecular formula: $C_{12}H_{21}N_5O_3$
Molecular weight: 283.33
CAS Registry No.: 64241-34-5
Merck Index: 1669

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 1.2 mL chloroform:EtOH 95:5, shake horizontally at 300 rpm for 12 min, centrifuge at 1220 g for 5 min. Remove the organic phase and add it to 100 μL 90 mM KCl in 10 mM HCl (pH 2), shake horizontally at 400 rpm for 12 min, centrifuge at 1220 g for 5 min, inject a 20 μL aliquot of the aqueous supernatant. (cf J. Chromatogr. 1984, 290, 223.)

HPLC VARIABLES

Column: 150 × 1 5 μm Nucleosil C18 in a glass-lined stainless steel column
Mobile phase: MeCN:100 mM NaH_2PO_4:1 M NaOH 18:79:3
Flow rate: 0.06
Injection volume: 20
Detector: UV 254 (2.4 μL flow cell)

CHROMATOGRAM

Retention time: 6
Internal standard: CGP 24 751
Limit of quantitation: 5 ng/mL

KEY WORDS

plasma; microbore

REFERENCE

Rouan,M.C. Microbore liquid chromatographic determination of cadralazine and cephalexin in plasma with large-volume injection, *J.Chromatogr.*, **1988**, *426*, 335–344.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Whole blood + 500 μL MeCN, mix, centrifuge at 2200 g for 2 min. Remove the supernatant and saturate it with anhydrous potassium carbonate (about 2 g per 1 mL of whole blood), centrifuge at 2200 g for 3 min, remove the MeCN layer, inject a 100 μL aliquot of the MeCN layer.

HPLC VARIABLES

Guard column: 10 μm octyl (Upchurch)
Column: 150 × 4.6 3 μm C8 (Alltech)
Mobile phase: MeCN:5 mM hexanesulfonic acid in 1% acetic acid 30:70
Flow rate: 1
Injection volume: 100
Detector: UV 250

CHROMATOGRAM

Retention time: 11
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: erythromycin, cimetidine, ranitidine, aspirin, acetaminophen, creatinine, theophylline, caffeine

KEY WORDS
whole blood; salting-out

REFERENCE
Rustum,A.M. Determination of cadralazine in human whole blood using reversed-phase high-performance liquid chromatography: utilizing a salting-out extraction procedure, *J.Chromatogr.*, **1989**, *489*, 345–352.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 g Plasma + 500 μL 1 μg/mL IS in 5 mM sulfuric acid + 1 mL water + 6 mL chloroform:EtOH 95:5, shake horizontally at 300 rpm for 12 min, centrifuge at 1220 g for 5 min. Remove the organic phase and add it to 500 μL pH 2 buffer, shake horizontally at 400 rpm for 12 min, centrifuge at 1220 g for 5 min, remove the aqueous layer and inject a 100 μL aliquot. Urine. 1 g Urine + 500 μL 60 μg/mL IS in 5 mM sulfuric acid + 1 mL pH 12 phosphate buffer + 6 mL chloroform:EtOH 95:5, shake horizontally at 300 rpm for 12 min, centrifuge at 1220 g for 5 min. Remove the organic phase and add it to 500 μL pH 2 buffer, shake horizontally at 400 rpm for 12 min, centrifuge at 1220 g for 5 min, remove the aqueous layer and inject a 100 μL aliquot. (pH 2 Buffer was 90 mmoles KCl in 1 L 10 mM HCl, pH 2.06. pH 12 Buffer was 3.8 g $Na_3PO_4.12H_2O$ in 1 L water adjusted to pH 12 with about 20 mL 2 M NaOH.)

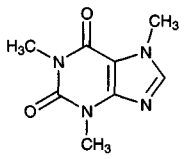
HPLC VARIABLES
Guard column: 50 × 3.2 30-40 μm Perisorb RP-8
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:100 mM pH 6 phosphate buffer 15:85 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 6 with about 30 mL 1 M NaOH.)
Column temperature: 30
Flow rate: 2.7
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: 2-[6-[ethyl(2-hydroxypropyl)amino]-3-pyridazinyl]hydrazine carboxylic acid propyl ester (CGP 24 751) (14)
Limit of quantitation: 10.59 nmole/g (urine), 0.141 nmole/g (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Hauffe,S.A.; Dubois,J.P. Determination of cadralazine in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *290*, 223–230.

Caffeine



Molecular formula: $C_8H_{10}N_4O_2$
Molecular weight: 194.19
CAS Registry No.: 58-08-2, 5743-12-4 (monohydrate)
Merck Index: 1674
Lednicer No.: 1 111

SAMPLE
Matrix: beverages
Sample preparation: Filter sample.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Hiasil C18 (Higgins)
Mobile phase: MeOH:25 mM phosphate buffer 45:55, pH 3.0
Flow rate: 1.0
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 2.0
Limit of detection: 1.2 mg/mL

OTHER SUBSTANCES
Simultaneous: aspartame, benzoic acid

KEY WORDS
comparison with UV spectrophotometry and capillary electrophoresis; soft drinks

REFERENCE
McDevitt,V.L.; Rodriguez,A.; Williams,K.R. Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, *J.Chem.Educ.*, **1998**, *75*, 625–629.

SAMPLE
Matrix: beverages, blood, formulations
Sample preparation: Plasma. 100 μL Plasma + 10 μL 1,3-dimethyl-7-(2-hydroxyethyl)xanthine in water, vortex, add 1 mL acetone, vortex for 1 min, centrifuge at 2500 g for 5 min. Filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Beverages. Dilute beverages 4-25 times with water, inject an aliquot. Tablets. Powder tablets, dissolve in water, filter, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 24
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)
Limit of detection: 140 ng/mL

OTHER SUBSTANCES
Simultaneous: hypoxanthine, pentoxifylline, propentofylline, theobromine, theophylline, uric acid, xanthine

KEY WORDS
plasma; tablets; pharmacokinetics

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

SAMPLE
Matrix: beverages, syrup
Sample preparation: Dilute syrup ten fold. Filter (0.45 μm) beverages and diluted syrup, inject a 10-20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 20:5:75
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 20 ng

OTHER SUBSTANCES
Simultaneous: acesulfame, benzoic acid, dulcin, p-hydroxybenzoic acid, saccharin, vanillin

REFERENCE
Veerabhadrarao,M.; Narayan,M.S.; Kapur,O.; Sastry,C.S. Reverse phase liquid chromatographic determination of some food additives, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 578–582.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL serum with 50 μL 1 M sodium hydroxide, extract with 1 mL 1.5 μ g/mL IS in isopropanol:chloroform 10:90. Centrifuge at 2000 g for 1 min, decant the organic layer, dry under a gentle air flow at 60°, dissolve the residue in 0.2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova Pak C18
Mobile phase: MeOH:THF:50 mM pH 4.7 ammonium acetate buffer 2:1.5:96.5
Flow rate: 1
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Internal standard: β-hydroxyethyltheophyline
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: theobromine, paraxanthine, theophyline

KEY WORDS
pharmakokinetics; serum

REFERENCE
Lee,T.C.; Charles,B.; Steer,P.; Flenady,V.; Shearman,A. Population pharmacokinetics of intravenous caffeine in neonates with apnea of prematurity, *Clin.Pharmacol.Ther.*, **1997**, *61*, 628–640.

SAMPLE
Matrix: blood
Sample preparation: Mix 50 μL plasma with 50 μL 20 μg/mL IS in water, vortex for 10 s, add 20 μL 20% perchloric acid, vortex for 10 s, centrifuge at 2000 g for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeCN:THF:concentrated acetic acid:water 2:2:0.5:95.5
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 7.5
Internal standard: 7-(β-hydroxypropyl)theophylline (9.2)
Limit of detection: 100 ng/mL
Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES
Extracted: theophylline
Simultaneous: β-hydroxyethyltheophylline, 8-chlorotheophylline, theobromine

KEY WORDS
plasma

REFERENCE
Schreiber-Deturmeny,E.; Bruguerolle,B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma, *J.Chromatogr.B*, **1996**, *677*, 305–312.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 5 μg/mL IS, extract with 6.0 mL dichloromethane, centrifuge. Aspirate aqueous layer to waste, evaporate organic layer under a stream of nitrogen at 35°. Reconstitute residue in 200 μL MeOH:water 15:85, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 C8 Rainin "Short One"
Mobile phase: Gradient. MeCN:MeOH:25 mM pH 3.0 phosphate buffer 3:15:82 to 15:15:70 over 10 min.
Detector: UV 273

CHROMATOGRAM
Retention time: 4.8
Internal standard: 7-(β-chloroethyl)theophylline (9.6)

KEY WORDS
plasma

REFERENCE
Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 11:89
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2.99

OTHER SUBSTANCES
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: Mix 250 μL serum with 250 μL 800 mM perchloric acid, vortex, centrifuge at 14000 g for 3-4 min. Remove 350 μL supernatant, adjust to ca. pH 5.0 with 27 μL 4 M NaOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Adsorbosphere ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:15 mM pH 4.9 potassium phosphate buffer 15:85 (After each elution flush the column with MeOH:water 80:20 for 5 min and then re-equilibrate with the for 5 min.)
Flow rate: 1.75
Injection volume: 100
Detector: UV 274

CHROMATOGRAM
Retention time: 17.75
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, aspirin, hydrocortisone, prednisolone, prednisone, phenylbutazone, phenytoin

KEY WORDS
serum

REFERENCE
Holland,D.T.; Godfredsen,K.A.; Page,T.; Connor,J.D. Simple high-performance liquid chromatography method for the simultaneous determination of serum caffeine and paraxanthine following rapid sample preparation, *J.Chromatogr.B*, **1998**, *707*, 105–110.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 0.2 mg/mL β-hydroxyethyltheophylline in buffer + 100 μL 40% aqueous trichloroacetic acid, vortex for 30 s, let stand for 5 min, centrifuge at 2000 g for 15 min, inject a 25 μL aliquot of the supernatant. (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeCN:buffer 6:94 (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)
Column temperature: 40
Flow rate: 2
Injection volume: 25
Detector: UV 274

CHROMATOGRAM
Retention time: 13
Internal standard: β-hydroxyethyltheophylline (9)

OTHER SUBSTANCES
Extracted: dyphylline, theophylline, theobromine

KEY WORDS
plasma

REFERENCE
Valia,K.H.; Hartman,C.A.; Kucharczyk,N.; Sofia,R.D. Simultaneous determination of dyphylline and theophylline in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *221*, 170–175.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 15 μg/mL β-hydroxyethyltheophylline in MeCN + 2 mL chloroform:isopropanol 95:5, mix for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5

Internal standard: β-hydroxyethyltheophylline (5.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: dyphylline, theophylline
Simultaneous: acetaminophen, aspirin, salicylic acid, procainamide, N-acetylprocainamide
Noninterfering: benzoic acid

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 200 μL 100 mM pH 7.0 phosphate buffer + 3 mL 0.5 μg/mL 8-chlorotheophylline in isopropanol, stir at 40000 rpm for 5 s using dental micromotor with a PTFE mixing head, centrifuge at 3500 g for 2 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 3.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:10 mM pH 5.2 sodium acetate buffer 6:3:91
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 12.1
Internal standard: 8-chlorotheophylline (8.85)

OTHER SUBSTANCES
Extracted: dyphylline, theophylline, proxyphylline, paraxanthine
Simultaneous: cefoxitin
Noninterfering: carbenicillin, cefoperazone, cephacetril, heparin, penicillin G, phenytoin, phenobarbital

KEY WORDS
plasma; serum; pharmacokinetics

REFERENCE
Wenk,M.; Eggs,B.; Follath,F. Simultaneous determination of diprophylline, proxyphylline and theophylline in serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *276*, 341–348.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 4 μg/mL 8-chlorotheophylline in MeCN, mix, centrifuge, evaporate the supernatant to dryness, reconstitute in 400 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 8 μm octadecyl CP-tm-Spher C18 glass column (Chrompack)
Mobile phase: MeCN:20 mM sodium acetate 20:80 adjusted to pH 4.4 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 10.0
Internal standard: 8-chlorotheophylline (8.8)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: theophylline

KEY WORDS
serum

REFERENCE
Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 1 mL reagent, vortex for 10 min, centrifuge for 2-3 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot. (Reagent was 1.5 mg β-hydroxyethyltheophylline and 25 μL glacial acetic acid in 100 mL chloroform:isopropanol 95:5.)

HPLC VARIABLES
Guard column: 30 mm long 5 μm Ultrasphere ion-pair
Column: 150 × 4.6 5 μm Ultrasphere ion-pair
Mobile phase: MeCN:MeOH:water:1 M tetra-n-butylammonium hydroxide 3.5:3.5:91:2 containing 1.82 g Trizma base (Tris, tris(hydroxymethyl)aminomethane), pH adjusted to 7.50 ± 0.03 with concentrated HCl
Flow rate: 1.2
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: k' 6.3
Internal standard: β-hydroxyethyltheophylline (k' 4.2)

OTHER SUBSTANCES
Extracted: theophylline, theobromine, 1,7-dimethyl xanthine
Simultaneous: acetaminophen, acetazolamide, allopurinol, dimethylurea, dyphylline, 3-methylxanthine, oxypurinol, procainamide, sulfadiazine, sulfamethazine, uric acid
Noninterfering: ampicillin, cefazolin, cephalothin, cephapirin, chlorotheophylline, 1,3-dimethyluric acid, gentamicin, lidocaine, methicillin, methylurea, 3-methyluric acid, quinidine, sulfamerazine, 1,3,7-trimethyluric acid

KEY WORDS
serum

REFERENCE
Lauff,J.J. Ion-pair high-performance liquid chromatographic procedure for the quantitative analysis of theophylline in serum samples, *J.Chromatogr.*, **1987**, *417*, 99–109.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 273

CHROMATOGRAM

Retention time: 2.18
Internal standard: 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental

Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin

Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS

serum

REFERENCE

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE

Matrix: blood

Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15

Detector: UV 208

CHROMATOGRAM

Retention time: 0.93
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS

plasma; SPE

REFERENCE

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE

Matrix: blood
Sample preparation: Inject 20 μL serum onto column A with mobile phase A and elute to waste, after 1 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 30 × 4.6 IRSP silica (for preparation see Anal. Chem. 1989, 61, 2445); B 150 × 4.6 TSK gel ODS-80TM
Mobile phase: A 20 mM NaH_2PO_4; B MeCN:100 mM NaH_2PO_4 10:90
Flow rate: A 0.8; B 1
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 12.5
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Extracted: theobromine, theophylline

KEY WORDS

serum; column-switching

REFERENCE

Haginaka,J.; Wakai,J.; Yasuda,H.; Kimura,Y. Determination of anticonvulsant drugs and methyl xanthine derivatives in serum by liquid chromatography with direct injection: column-switching method using a new internal-surface reversed-phase silica support as a precolumn, *J.Chromatogr.*, **1990**, *529*, 455–461.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 25 μg/mL lidocaine in 4 mM HCl, mix, add 100 μL 1 M pH 9.0 borate buffer, add 1 mL chloroform:EtOH 82.5:17.5, mix, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:buffer 12:16:72 (Buffer was 31 mM sodium acetate adjusted to pH 5.1 with 40% phosphoric acid containing 0.15 mM tetrabutylammonium phosphate.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5
Internal standard: lidocaine (10)
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: cocaine
Simultaneous: barbital, phenobarbital, flumazepil, mazindol, hexobarbital
Noninterfering: amphetamine, desipramine, tetracaine, methadone, reserpine, buspirone, diazepam, haloperidol, chlordiazepoxide, oxazepam, midazolam, clonazepam, chlorpromazine, pentobarbital
Interfering: nicotine, procaine, cotinine

KEY WORDS
serum; rat

REFERENCE
Lau,C.E.; Ma,F.; Falk,J.L. Simultaneous determination of cocaine and its metabolites with caffeine in rat serum microsamples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *532*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or serum + 35 μL 22 μg/mL 3-ethylxanthine in 20 mM pH 4.0 acetate buffer, add to a Celute-MX SPE cartridge (Jones Chromatography), let stand for 10 min, elute with two portions of isopropanol:dichloromethane 10:90, evaporate the eluate to dryness under a stream of nitrogen below 37°, reconstitute the residue in 100 μL mobile phase B, centrifuge at 4400 rpm in a refrigerated centrifuge for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 3 μm ODS Apex I (Jones Chromatography)
Mobile phase: Gradient. A was MeCN:THF:10 mM pH 4.0 acetate buffer 25:2:73. B was THF:10 mM pH 4.0 acetate buffer 0.01:99.99. From A:B 0:100 increasing at 2.1% A/min.
Column temperature: 50
Flow rate: 0.8
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 20.17
Internal standard: 3-ethylxanthine (13.64)

OTHER SUBSTANCES
Simultaneous: theophylline, theobromine, paraxanthine, methylxanthines, dimethyluric acids, trimethyluric acid, acetaminophen

KEY WORDS
plasma; serum; SPE

REFERENCE
Leakey,T.E. Simultaneous analysis of theophylline, caffeine and eight of their metabolic products in human plasma by gradient high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *507*, 199–220.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL 220 μg/mL 4-nitroacetanilide in ethyl acetate, vortex for 30 s, centrifuge at 7000 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: Isopropanol:100 mM pH 5.0 sodium acetate buffer 2:98
Flow rate: 2
Injection volume: 10
Detector: UV 278

CHROMATOGRAM
Retention time: 3.85
Internal standard: 4-nitroacetanilide (8.27)
Limit of quantitation: 10000 ng/mL

OTHER SUBSTANCES
Extracted: chloramphenicol
Simultaneous: theobromine, theophylline, diphylline, chloramphenicol 3-monosuccinate
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, carbamazepine, cyclosporine, digoxin, desipramine, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum

REFERENCE
Markin,R.S.; Wadman,M.C.; Bottjen,P.L.; Haven,M.C.; Huth,J.A. Short-column liquid chromatographic assay for caffeine and chloramphenicol in serum, *J.Chromatogr.*, **1990**, *525*, 464–470.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm internal surface reversed phase Pinkerton, silica derivatized with glycine-phenylalanine-phenylalanine (Regis) (periodically reverse the column)
Mobile phase: 100 mM pH 6.8 phosphate buffer
Flow rate: 0.3
Injection volume: 10

Detector: UV 275

CHROMATOGRAM
Retention time: 13.03
Limit of detection: <1000 ng/mL

OTHER SUBSTANCES
Extracted: dyphylline, doxofylline, theophylline
Noninterfering: acetaminophen, amitriptyline, amphetamine, atropine, benzoylecgonine, benztropine, caffeine, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, chlorprothixene, cimetidine, cocaine, codeine, dextromethorphan, diazepam, diphenhydramine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, flurazepam, glutethimide, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, nicotine, nordiazepam, nortriptyline, orphenadrine, papaverine, pentazocine, phenacetin, phencyclidine, phenmetrazine, phenolphthalein, phentermine, phenylpropanolamine, phenytoin, prazepam, procainamide, procaine, propoxyphene, propranolol, protriptyline, pseudoephedrine, pyrilamine, quinine, salicylamide, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, trimethobenzamide, trimethoprim, tripelennamine

KEY WORDS
plasma; serum; direct injection

REFERENCE
Tagliaro,F.; Dorizzi,R.; Frigerio,A.; Marigo,M. Non-extraction HPLC method for simultaneous measurement of dyphylline and doxofylline in serum, *Clin.Chem.*, **1990**, *36*, 113–115.

SAMPLE
Matrix: blood
Sample preparation: 10 µL Plasma + 300 µL 100 mM pH 6.0 KH_2PO_4 buffer + 100 µL 10 µg/mL diprophylline + 2 mL chloroform:isopropanol 50:50, vortex for 30 s, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100 µL mobile phase, inject a 25 µL aliquot.

HPLC VARIABLES
Column: NovaPak C18 radial compression
Mobile phase: MeOH:MeCN:10 mM KH_2PO_4 9:2.5:90
Flow rate: 2
Injection volume: 25
Detector: UV 270

CHROMATOGRAM
Retention time: 17
Internal standard: diprophylline (10)

OTHER SUBSTANCES
Extracted: theophylline, 3-methylxanthine, theobromine

KEY WORDS
plasma

REFERENCE
Augustijns,P.; Verbeke,N. A microassay method for the determination of theophylline in biological samples using HPLC with electrochemical detection, *J.Liq.Chromatogr.*, **1992**, *15*, 1303–1313.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 20 μg/mL hydroxyethyltheophylline in 2 M perchloric acid, vortex, centrifuge 5 min, inject 50 μL aliquot of supernatant.

HPLC VARIABLES
Column: 125 × 4 LiChroSpher RP-8 5 μm
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mL 2 M sodium acetate + 845 mL water, pH adjusted to 4.0 with acetic acid.)
Column temperature: 45
Flow rate: 1.5
Injection volume: 50
Detector: UV 282

CHROMATOGRAM
Retention time: 9.6
Internal standard: hydroxyethyltheophylline (5.6)

OTHER SUBSTANCES
Simultaneous: theophylline, chloramphenicol

KEY WORDS
serum

REFERENCE
Hannak,D.; Haux,P.; Scharbert,F.; Kattermann,R. Liquid chromatographic analysis of phenobarbital, phenytoin, and theophylline, *Wien.Klin.Wochenschr.Suppl.*, **1992**, *191*, 27–31.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 3 μg/mL 8-chlorotheophylline in mobile phase + 100 μL 1 M HCl + 3 mL dichloromethane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM NaH_2PO_4 30:70
Flow rate: 0.8
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 8
Internal standard: 8-chlorotheophylline (11.7)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, theobromine, paraxanthine, metabolites
Noninterfering: acetaminophen, phenylbutazone, phenacetin, thiamine, salicylic acid, phenobarbital, chlorpheniramine, trimethadione, hydrocortisone, prednisolone, prednisone, phenytoin, aspirin, ethenzamide

KEY WORDS
plasma

REFERENCE

Tanaka,E. Simultaneous determination of caffeine and its primary demethylated metabolites in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 311–314.

SAMPLE

Matrix: blood

Sample preparation: Dilute serum with an equal volume 7.5 μg/mL theobromine, inject a 20 μL aliquot directly.

HPLC VARIABLES

Column: 150 × 4.6 ChromSpher 5 BioMatrix (Chrompack)
Mobile phase: MeCN:water 5:95
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 4.2
Internal standard: theobromine (3.3)

OTHER SUBSTANCES

Simultaneous: theophylline

KEY WORDS

serum

REFERENCE

Helmsing,P.J.; Huisman,R.; van der Weele,A. HPLC determination of caffeine and theophylline by direct serum injection [letter], *Clin.Chem.*, **1993**, *39*, 1348–1349.

SAMPLE

Matrix: blood

Sample preparation: Condition an Merck Extrelut-3 glass extraction column with 12 mL dichloromethane the day before. 1.5 mL Serum + 100 μL 3 μg/mL N-ethylnorcotinine in water + 1.4 mL 0.5 M NaOH. Add to column, after 15 min elute under gravity with dichloromethane:isopropanol 9:1. Add 300 μL 25 mM HCl in MeOH to the organic phase and evaporate it to dryness under nitrogen. Redissolve in 100 μL water and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC8DB

Mobile phase: Gradient. A was MeCN:water 3.6:96.4 containing 2 mL/L triethylamine, 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, 12 mM citric acid adjusted to pH 4.7 with citric acid. B was MeCN:water 19.7:80.3 containing 2 mL/L triethylamine, 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, 12 mM citric acid adjusted to pH 5.2 with citric acid. A:B 100:0 for 15 min then to 50:50 over 20 min using a concave gradient. Re-equilibrate for 15 min before next injection.

Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 27
Internal standard: N-ethylnorcotinine (33)

OTHER SUBSTANCES

Simultaneous: nicotine, cotinine-N-oxide, trans-3'-hydroxycotinine, norcotinine, cotinine

KEY WORDS
serum

REFERENCE
Zuccaro,P.; Altieri,I.; Rosa,M.; Passa,A.R.; Pichini,S.; Ricciarello,G.; Pacifici,R. Determination of nicotine and four metabolites in the serum of smokers by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *621*, 257–261.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5
Limit of detection: 0.10 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS
whole blood

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE
Matrix: blood
Sample preparation: Wash PCPure cartridge containing 0.4 g hydroxyapatite with 10 mL MecN and remove MeCN by evaporation. 75 μL Plasma + 25 μL of 50 μg/mL IS in 0.5% aqueous MeCN injected onto PCPure cartridge, elute with MeCN:water 10:90. Use first 600 μL of eluate, inject 20 μL aliquots.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 5:95
Column temperature: 40
Flow rate: 1
Injection volume: 20

Detector: UV 280

CHROMATOGRAM
Retention time: 15
Internal standard: 7-(2-hydroxyethyl)theophylline (10)

OTHER SUBSTANCES
Simultaneous: theophylline

KEY WORDS
plasma; SPE

REFERENCE
Iwase,H.; Gondo,K.; Koike,T.; Ono,I. Novel precolumn deproteinization method using a hydroxyapatite cartridge for the determination of theophylline and diazepam in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1994**, *655*, 73–81.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL theophylline + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:0.5 mM phosphoric acid 10:90
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Internal standard: theophylline

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 25 μL 100 μg/mL theophylline, extract with chloroform:isopropanol 85:15. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 microsphere C18 (Chrompack)
Mobile phase: MeCN:55 mM pH 4.0 sodium acetate buffer 6:94
Flow rate: 1.2
Detector: UV 278

CHROMATOGRAM
Internal standard: theophylline

KEY WORDS
plasma; pig; pharmacokinetics

REFERENCE
Monshouwer,M.; Witkamp,R.F.; Nijmeijer,S.M.; Pijpers,A.; Verheijden,J.H.M.; Van Miert,A.S.J.P.A.M. Selective effects of a bacterial infection (*Actinobacillus pleuropneumoniae*) on the hepatic clearance of caffeine, antipyrine, paracetamol, and indocyanine green in the pig, *Xenobiotica*, **1995**, *25*, 491–499.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: MeCN:50 mM pH 6.9 phosphate buffer 1.8:98.2
Flow rate: 0.6
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: theophylline

KEY WORDS
serum; direct injection

REFERENCE
Nimura,N.; Itoh,H.; Kinoshita,T. Diol-bonded silica gel as a restricted access packing forming a binary-layered phase for direct injection of serum for the determination of drugs, *J.Chromatogr.A*, **1995**, *689*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 70% perchloric acid, centrifuge at 2000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC8DB
Mobile phase: MeCN:MeOH:THF:buffer 4.3:2.3:0.3:93.1 (Buffer was 2 mL/L triethylamine containing 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, and 12 mM citric acid, adjusted to pH 4.7 with citric acid.)
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: nicotine, cotinine

KEY WORDS
serum

REFERENCE
Pichini,S.; Altieri,I.; Passa,A.R.; Rosa,M.; Zuccaro,P.; Pacifici,R. Use of solvent optimization software for rapid selection of conditions for reversed-phase high-performance liquid chromatography of nicotine and its metabolites, *J.Chromatogr.A*, **1995**, *697*, 383–388.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 3.06
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; tri-

fluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 6.16

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 6.1
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, gastric contents, pancreatic juice
Sample preparation: 500 μL Serum, pancreatic juice, or gastric juice + 25 μL 200 μg/mL β-hydroxyethyltheophylline in water+ 500 μL MeCN, centrifuge at 6000 rpm for 10 min, remove supernatant and add it to 1.8 mL chloroform, vortex, centrifuge at 6000 rpm for 10 min. Remove the lower organic phase and evaporate it to dryness at 60° under a stream of air, dissolve the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: C18 LichroCART
Column: 250 × 4.6 7 μm Lichrosorb C18
Mobile phase: THF:MeOH:10 mM pH 3.5 KH_2PO_4 1:20:79
Flow rate: 0.8
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 10.78
Internal standard: β-hydroxyethyltheophylline (7.48)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: theophylline, 1-methyluric acid, 1-methylxanthine, theobromine, paraxanthine

KEY WORDS

serum; dog

REFERENCE
Casoli,P.; Vérine,H. High performance liquid chromatographic determination of methylxanthines in canine serum, gastric and pancreatic juices, *Biomed.Chromatogr.*, **1990**, *4*, 209–213.

SAMPLE
Matrix: blood, milk
Sample preparation: 25 μL serum + 100 μL 250 ng/mL β-hydroxyethyltheophylline in water + 200 μL 200 mM pH 6.0 phosphate buffer + 3 mL dichloromethane, shake at 120 oscillations/min for 20 min, centrifuge at 174 g. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 230 μL water, heat at 90° for 6 min, vortex, inject a 180 μL aliquot. (No details given for milk extraction.)

HPLC VARIABLES
Guard column: Corasil Bondapak C18
Column: 5 μm radial-compression C18 (Waters)
Mobile phase: MeOH:THF:10 mM KH_2PO_4 9:1:90 adjusted to pH 3.5
Flow rate: 1.2
Injection volume: 180
Detector: UV 214

CHROMATOGRAM
Retention time: 16.5
Internal standard: β-hydroxyethyltheophylline (10.9)

OTHER SUBSTANCES
Extracted: theophylline, paraxanthine, theobromine

KEY WORDS
serum; pharmacokinetics

REFERENCE
Oo,C.Y.; Burgio,D.E.; Kuhn,R.C.; Desai,N.; McNamara,P.J. Pharmacokinetics of caffeine and its metabolites in lactation: Prediction of milk to serum concentration ratios, *Pharm.Res.*, **1995**, *12*, 313–316.

SAMPLE
Matrix: blood, saliva
Sample preparation: Add 500 μL plasma, serum, or saliva to 200 mg ammonium sulfate, add 50 μL 15 μg/mL IS in water, add 500 μL 200 mM pH 4.5 sodium acetate buffer, vortex briefly, add 3 mL dichloromethane, shake at 85 cycles/min for 10 min, centrifuge at 2000 g for 10min. Evaporate the organic layer to dryness at 40° under a stream of nitrogen, reconstitute in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: 100 × 4.6 3 μm Microsorb MV C18
Mobile phase: MeOH:THF:100 mM pH 4.5 sodium acetate:water 6.5:1.4:10:82.1
Flow rate: 0.8
Injection volume: 50
Detector: UV 274

CHROMATOGRAM
Retention time: 9.1
Internal standard: β-hydroxyethyltheophylline (6.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, paraxanthine, theobromine, theophylline

Noninterfering: chlorzoxazone, dapsone

KEY WORDS
plasma; serum

REFERENCE
Frye,R.F.; Stiff,D.D.; Branch,R.A. A sensitive method for the simultaneous determination of caffeine and its dimethylxanthine metabolites in human plasma: Application to CYP1A2 phenotyping, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1161–1171.

SAMPLE
Matrix: blood, saliva
Sample preparation: Saliva. Add 3 mg N-acetylcysteine (mucolytic agent) to 200 μL saliva), let stand for 10-15 min. 50 μL Saliva + 50 μL 1 M NaOH, extract with 1 mL 10% (v/v) isopropanol in chloroform (Caution! Chloroform is a carcinogen!) containing 1.5 mg/L β-hydroxyethyl theophylline. Centrifuge at 2000 g for 1 min. Evaporate organic layer to dryness under a gentle airflow at 60°. Dissolve residue in 200 μL mobile phase, inject a 50 μL aliquot. Serum. 50 μL Serum + 50 μL 1 M NaOH, extract with 1 mL 10% isopropanol in chloroform containing 1.5 mg/L β-hydroxyethyl theophylline. Centrifuge at 2000 g for 1 min. Evaporate organic layer to dryness under a gentle airflow at 60°. Dissolve the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 C18 Nova Pak
Mobile phase: MeOH:THF:50 mM pH 4.7 ammonium acetate buffer 2:1.5:96.5
Flow rate: 1
Injection volume: 50

CHROMATOGRAM
Retention time: 10.2
Internal standard: β-hydroxyethyl theophylline (6.6)
Limit of quantitation: 200 ng/L

OTHER SUBSTANCES
Extracted: paraxanthine, theobromine, theophylline

KEY WORDS
serum

REFERENCE
Lee,T.C.; Charles,B.G.; Steer,P.A.; Flenady,V.J. Saliva as a valid alternative to serum in monitoring intravenous caffeine treatment for apnea of prematurity, *Ther.Drug Monit.*, **1996**, *18*, 288–293.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize brain in twenty volumes of water, centrifuge at 1500 g, freeze at -20° for 2 h, centrifuge. 200 μL Supernatant + 100 μL pH 7.2 phosphate buffer, mix, add 6 mL dichloromethane:propanol 95:5, mix for 2 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 25 μL aliquot. Serum. 200 μL Serum + 100 μL pH 7.2 phosphate buffer, mix, add 6 mL dichloromethane:propanol 95:5, mix for 2 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak C18

Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: THF:10 mM Na_2HPO_4 3:97, pH adjusted to 6.5 with phosphoric acid
Flow rate: 2.5
Injection volume: 25
Detector: UV 273

CHROMATOGRAM
Retention time: 7
Limit of detection: 62.5 ng/mL (serum), 62.5 ng/g (tissue)

OTHER SUBSTANCES
Extracted: theophylline, theobromine, paraxanthine

KEY WORDS
serum; rat; brain

REFERENCE
Parra,P.; Limon,A.; Ferre,S.; Guix,T.; Jane,F. High-performance liquid chromatographic separation of caffeine, theophylline, theobromine and paraxanthine in rat brain and serum, *J.Chromatogr.*, **1991**, *570*, 185–190.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. 100 μL Urine + 100 μL 1 M NaOH, let stand at room temperature for 10 min, add 100 μL 1 M HCl, make up to 1 mL with mobile phase, vortex, centrifuge at 13000 g for 2 min, inject a 20 μL aliquot. Plasma. 500 μL Plasma + 100 μL 60% perchloric acid, add 200 μL 1 M NaOH to make pH >9, let stand for 10 min, add 200 μL 1 M HCl, add 200 μL 50 μg/mL β-hydroxyethyltheophylline, add 6 mL chloroform: isopropanol 50:50, agitate for 15 min on a rotating mixer, centrifuge at 4000 g. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.5 5 μm ChromSep (Chrompack)
Mobile phase: THF:10 mM sodium acetate 0.8:99.2
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.03
Internal standard: β-hydroxyethyltheophylline (4.76)
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Extracted: 1,3,7-trimethyluric acid, 1,7-dimethylxanthine, 1,3-dimethylxanthine, metabolites

KEY WORDS
plasma

REFERENCE
Dobrocky,P.; Bennett,P.N.; Notarianni,L.J. Rapid method for the routine determination of caffeine and its metabolites by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *652*, 104–108.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 6.647

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 14.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE

Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE

Matrix: dialysate

Sample preparation: Dialyze blood with Ringer's solution, inject a 0.5 μL aliquot of the dialysate.

HPLC VARIABLES

Column: 14 × 1 3 μm BAS Sep-Stik ODS (Bioanalytical Systems)
Mobile phase: MeCN:50 mM pH 2.5 ammonium phosphate buffer 5:95
Flow rate: 0.2
Injection volume: 0.5
Detector: UV 273

CHROMATOGRAM

Retention time: 0.77
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, theobromine, paraxanthine

KEY WORDS

rat; microbore

REFERENCE

Chen,A.; Lunte,C.E. Microdialysis sampling coupled on-line to fast microbore liquid chromatography, *J.Chromatogr.A*, **1995**, *691*, 29–35.

SAMPLE

Matrix: food

Sample preparation: Dilute 5 mL beverage with 5 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil PXS 5/25 ODS-3 or 150 × 4.6 5μm Ultrasphere ODS
Mobile phase: MeOH:water:33% acetic acid 50:49:1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5
Internal standard: aspirin (6.6)

KEY WORDS

drink; beverage; soft drink; tea; coffee

REFERENCE

Galasko,G.T.F.; Furman,K.I.; Alberts,E. The caffeine content of non-alcoholic beverages, *Food Chem.Toxicol.*, **1989**, *27*, 49–51.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets or capsules, weigh 20 mg, add 100 (tablets) or 500 (capsules) mL 50 mM sodium dodecyl sulfate, sonicate for 3 min, filter (sintered glass 4), dilute to a concentration of ca. 6 μg/mL with 50 mM sodium dodecyl sulfate, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 5 μm Spherisorb ODS-2 C18
Column: 120 × 4.5 μm Spherisorb ODS-2 C18
Mobile phase: Isopropanol:50 mM sodium dodecyl sulfate 1.5:98.5, pH 7.0
Flow rate: 1
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 11.5

KEY WORDS
tablets; capsules

REFERENCE
Perez-Martinez,I.; Sagrado,S.; Medina-Hernández,M.J. Chromatographic determination of caffeine in pharmaceutical formulations using micellar mobile phases, *Chromatographia*, **1996**, *43*, 149–152.

SAMPLE
Matrix: formulations
Sample preparation: Add 50 mL of mobile phase to 0.5 g of sample and swirl to aid dissolution. Dilute to 100 mL with mobile phase. Dilute 1:10, filter (0.22 μm nylon). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:water:triethylamine:acetic acid 5.5:94.1:0.2:0.2
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2.2
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Weigh 500 mg homogenized analgesic powder, transfer to 100 mL volumetric flask, add ca. 50 mL mobile phase, swirl and dilute to volume with mobile phase. Dilute an aliquot of this solution 1:10 with mobile phase, filter (0.20 μm Cameo nylon filter, MSI, Westboro, MA) an aliquot, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:triethylamine:acetic acid:water 5.5:0.2:0.2:94.1 (Prepare mobile phase as follows. Mix 110 mL MeCN, 4 mL triethylamine, 4 mL glacial acetic acid and make up to 2 L with water.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Extracted: aspirin, acetaminophen
Noninterfering: salicylic acid

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau Science, Spain)
Mobile phase: MeOH:20 mM pH 4.0 KH_2PO_4 30:70 adjusted to pH 4.0 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 135
Detector: UV 274

CHROMATOGRAM
Retention time: 6.6
Limit of quantitation: 2.2 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin, salicylic acid, thiamine

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous).

Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 2.6
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate
Interfering: aspirin, formebolone, testolactone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: biotin, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, 1993, **1993**,

SAMPLE
Matrix: formulations

Sample preparation: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM
Retention time: 10.40
Limit of quantitation: 3 μg/mL

OTHER SUBSTANCES
Simultaneous: methamphetamine, amphetamine, ephedrine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder half a tablet, add 9 mL mobile phase, sonicate for 20 min, make up to 10 mL with mobile phase, filter (Whatman type 40 and 0.2 μm Millipore), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 CN
Mobile phase: MeCN:THF:buffer 7:6:87 (Buffer was 0.8% acetic acid containing 5 mM sodium hexanesulfonate, 10 mM di-n-butylamine, and 0.12% phosphoric acid, pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 298

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 1.1 μg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen (UV 310), chlorpheniramine (UV 265), guaifenesin (glycerylguaicolate) (UV 284), phenylpropanolamine (UV 260)

KEY WORDS
tablets

REFERENCE
Indrayanto,G.; Sunarto,A.; Adriani,Y. Simultaneous assay of phenylpropanolamine hydrochloride, caffeine, paracetamol, glycerylguaiacolate and chlorpheniramine in Silabat™ tablet using HPLC with diode array detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1555–1559.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder tablets, weigh out amount equivalent to 20 mg enalapril maleate, suspend in 100 mL mobile phase, filter, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 12 μm Hypersil C18
Mobile phase: MeCN:water 20:80 adjusted to pH 3.8 with acetic acid
Flow rate: 1
Injection volume: 5
Detector: UV 215 for 3.5 min, then UV 275

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: enalapril, hydrochlorothiazide

KEY WORDS
tablets

REFERENCE
el Walily,A.F.M.; Belal,S.F.; Heaba,E.A.; El Kersh,A. Simultaneous determination of enalapril maleate and hydrochlorothiazide by first-derivative ultraviolet spectrophotometry and high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 851–856.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out powdered sample containing 8 mg caffeine, add 80 mL MeOH, sonicate for 10 min, dilute to 100 mL with MeOH, centrifuge. Remove a 5 mL aliquot of the supernatant and add it to 1 mL 2 mg/mL resorcinol, add 2 mL MeOH, make up to 20 mL with 50 mM pH 3.0 triethylamine phosphate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: THF:50 mM pH 3.0 triethylamine phosphate 12:88
Flow rate: 0.6
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 15
Internal standard: resorcinol (9)

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin (post-column irradiation gives an increase in peak height), propyphenazone

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 1 mL chloroform:isopropanol 85:15 (Caution! Chloroform is a carcinogen!). Add 25 pmole 4-acetoimidophenol. (Metabolites

were extracted with the addition of an additional 3.5 mL chloroform:isopropanol 85:15 and 500 mg ammonium sulfate before centrifugation.). Mix, centrifuge at 3000 rpm for 10 min. Remove the organic phase, evaporate it and reconstitute the residue with 150 μL mobile phase A. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Beckman C18 (Phenomenex, USA)
Mobile phase: Gradient. A was THF:50 mM pH 4.5 sodium acetate 1:99. B was MeCN:50 mM pH 4.5 sodium acetate 60:40. A:B 100:0 for 1 min, to 70:30 over 23 min, to 0:100 over 1 min, maintain at 0:100 for 5 min, to 100:0 over 1 min, maintain at 100:0 for 9 min.
Flow rate: 0.8
Detector: UV 270

CHROMATOGRAM
Retention time: 18
Internal standard: 4-acetoimidophenol (16)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Ring,B.J.; Catlow,J.; Lindsay,T.J.; Gillespie,T.; Roskos,L.K.; Cerimele,B.J.; Swanson,S.P.; Hamman,M.A.; Wrighton,S.A. Identification of the human cytochromes P450 responsible for the in vitro formation of the major oxidative metabolites of the antipsychotic agent olanzapine, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 658–666.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 275 μL Microsomal incubation + 50 μL 30% perchloric acid, centrifuge at 2000 g for 10 min, inject a 60 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Supelcosil LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:THF:20 mM pH 3.5 sodium perchlorate 0.5:0.5:99
Flow rate: 1.5
Injection volume: 60
Detector: UV 280

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Extracted: paraxanthine, theobromine, theophylline, trimethylurate

KEY WORDS
monkey; liver

REFERENCE
Bullock,P.; Pearce,R.; Draper,A.; Podval,J.; Bracken,W.; Veltman,J.; Thomas,P.; Parkinson,A. Induction of liver microsomal cytochrome P450 in cynomolgus monkeys, *Drug Metab.Dispos.*, **1995**, *23*, 736–748.

SAMPLE
Matrix: milk
Sample preparation: Centrifuge at 12800 g for 10 min, remove top layer of fat with a spatula. 450 μL Supernatant + 50 μL water + 150 μL 21.65 μg/mL proxyphylline in 6% perchloric acid, vortex for 5 s, cool on ice for 10-15 min, centrifuge at 12800 g for 10 min, inject a 50 μL aliquot of the supernatant

HPLC VARIABLES
Guard column: Co:Pell ODS glass beads (Whatman)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 10 mM sodium acetate + 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 4.9 with 2 M NaOH. B was MeOH:water 50:50 containing 10 mM sodium acetate + 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 4.8 with glacial acetic acid. A:B 100:0 for 7.5 min, then to 85:15 over 7.5 min, then to 70:30 over 10 min, then to 68:32 over 4 min, then to 100:0 over 3 min
Flow rate: 1.5
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 30
Internal standard: proxyphylline (25)

OTHER SUBSTANCES
Simultaneous: theobromine, paraxanthine, theophylline

REFERENCE
Blanchard,J.; Weber,C.W.; Shearer,L.E. HPLC analysis of methylxanthines in human breast milk, *J.Chromatogr.Sci.*, **1990**, *28*, 640–642.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 18:82
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 273

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 1.66

OTHER SUBSTANCES
Simultaneous: levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, fenethyline, phendimetrazine, methylphenidate, phenelzine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeCN:water 80:20, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3057 ODS silica (Hitachi)
Mobile phase: MeCN:water 25:75
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: dipyrone (sulpyrin), guaifenesin (guaiacol glycerol ether), acetaminophen, bucetin (3-hydroxy-p-butyrophenetidine), methyl p-hydroxybenzoate, phenacetin

KEY WORDS
semi-micro

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 25:1.5:0.5:73
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: theobromine, diphylline, theophylline, 8-chlorotheophylline

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 1.329

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 225, 267

CHROMATOGRAM
Retention time: 1.5
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions

Sample preparation: Dilute a 10 mg/mL solution of caffeine in water 1:1000 with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 2540 × 4.6 5 μm C18 (Supelco)

Mobile phase: MeOH:10 mM ammonium acetate and 2.5 mM sodium heptanesulfonate 20:80, pH adjusted to 5.1 with glacial acetic acid

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 10.5

OTHER SUBSTANCES

Simultaneous: benzoic acid

KEY WORDS

stability-indicating; water

REFERENCE

Donnelly,R.F.; Tirona,R.G. Stability of citrated caffeine injectable solution in glass vials, *Am.J.Hosp.Pharm.*, **1994**, *51*, 512–514.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheni-

ramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.79 (A), 3.84 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 mm long 5 μm Microsorb-MV C18
Mobile phase: MeOH:water 60:40
Flow rate: 1
Detector: UV 270

REFERENCE

Phillips,C.A.; Michniak,B.B. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers, *J.Pharm.Sci.*, **1995**, *84*, 1427–1433.

SAMPLE

Matrix: urine
Sample preparation: 40 μL Urine + 100 μL 160 μM IS in 30% EtOH, make up to 400 μL with 10 mM pH 4.0 acetate buffer. Add 5 mL ethyl acetate:2-propanol 93:7, shake for 10

min, centrifuge at 1000 g for 10 min, freeze the aqueous phase at -30°. Evaporate organic phase to dryness under a stream of nitrogen at 55°. Reconstitute the residue in 300 μL mobile phase, vortex for 10 s, centrifuge at 1000 g for 30 s, inject a 40 μL aliquot. (Keep sample in the autosampler at 5°.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:10 mM pH 4.0 acetate buffer 9:91
Column temperature: 30
Flow rate: 1 from 0 to 11 min, 1.5 from 11 to 17 min, 2.5 from 17 to 30 min
Injection volume: 40
Detector: UV 273

CHROMATOGRAM
Retention time: 25
Internal standard: β-hydroxy-ethyl theophylline (19.8)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics

REFERENCE
Rasmussen,B.B.; Brosen,K. Determination of urinary metabolites of caffeine for the assessment of cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activity in humans, *Ther.Drug Monit.*, **1996**, *18*, 254–262.

SAMPLE
Matrix: urine
Sample preparation: Add 100-120 mg NaCl, 50 μL 100 μg/mL β-hydroxyethyltheophylline, and 100 μL ammonia buffer to 1 mL urine. Extract with 5 mL MeOH:dichloromethane 10:90 for 10 min, centrifuge at 150 g for 5 min, remove the upper aqueous layer and evaporate the organic layer under nitrogen at 40°. Dissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot. (Prepare the pH 9.5 ammonia buffer by adding ammonia to a saturated ammonium chloride solution.)

HPLC VARIABLES
Guard column: 10 × 2 40 μm C18
Column: 100 × 3 5 μm Hypersil 5 ODS (Chrompack)
Mobile phase: THF:water 1:100
Flow rate: 1
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 6.5
Internal standard: β-hydroxyethyltheophylline (4.5)

OTHER SUBSTANCES
Extracted: theophylline, theobromine, paraxanthine

KEY WORDS
horse; human; urine

REFERENCE
Delbeke,F.T.; De Backer,P. Threshold level for theophylline in doping analysis, *J.Chromatogr.B*, **1996**, *687*, 247–252.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 0.7

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone

Interfering: cotinine, benzoylecgonine, secobarbital

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 270

CHROMATOGRAM

Retention time: 10.3

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 2.8

Internal standard: 7-propyltheophylline (4.5)

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE
Matrix: urine
Sample preparation: Urine. Stabilize urine with 1 M pH 3.0 citric acid phosphate buffer. 50 μL Urine + 120 mg ammonium sulfate + 50 μL 1.2 mg/mL acetaminophen + 6 mL chloroform:isopropanol 95:5, shake gently for 20 min, centrifuge at 2000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL 0.05% acetic acid, inject a 50 μL aliquot. Plasma. 500 μL Plasma + 75 μL 1 M HCl + 25 μL 500 μg/mL acetanilide + 120 mg ammonium sulfate + 6 mL chloroform: isopropanol 95:5, shake gently for 20 min, centrifuge at 2000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL 0.05% acetic acid, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeOH:0.25% acetic acid 7.5:92.5 for 10 min then to 21:79 over 20 min, maintain at 21:79 for 5 min, then to 60:40 over 5 min, return to 7.5:92.5 over 5 min, re-equilibrate for 15 min. (urine) Isocratic. MeOH:0.25% acetic acid 20:80. (plasma)
Flow rate: 1.2
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Internal standard: acetaminophen (urine), acetanilide (plasma)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Rost,K.L.; Roots,I. Accelerated caffeine metabolism after omeprazole treatment is indicated by urinary metabolite ratios: Coincidence with plasma clearance and breath test, *Clin.Pharmacol.Ther.*, **1994**, *55*, 402–411.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 10-fold with 5 μg/mL β-hydroxyethyltheophylline in water, mix, centrifuge at 14000 rpm for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 40 × 2.5 10 μm Lichrosorb RP-2
Column: 150 × 4.6 5 μm Ultrasphere-ODS
Mobile phase: MeCN:THF:10 mM sodium acetate 3:0.1:96.9 containing 5 mM tetrabutylammonium hydrogen sulfate, pH 4.7
Flow rate: 1.5
Injection volume: 25
Detector: UV 280

CHROMATOGRAM
Retention time: 18
Internal standard: β-hydroxyethyltheophylline (11)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: metabolites, 1,3-dimethyluric acid, 1-methyluric acid, 3-methylxanthine, theophylline

REFERENCE
Tajerzadeh,H.; Dadashzadeh,S. An isocratic high-performance liquid chromatographic system for simultaneous determination of theophylline and its major metabolites in human urine, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1507–1512.

SAMPLE
Matrix: urine
Sample preparation: Condition a 700 mg Extrelut-1 diatomaceous earth glass SPE cartridge with 6 mL dichloromethane, let stand for 1 day. 200 μL Urine + 200 μL 10 μg/mL N-ethylnornicotine in water + 600 μL 500 mM NaOH, add to the SPE cartridge, let stand for 10 min, elute with 5 mL dichloromethane:isopropanol 90:10. Add the eluate to 100 μL 25 mM HCl in MeOH and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC8DB (Supelchem)
Mobile phase: MeCN:water 9:80 containing 5 mL/L triethylamine, 670 mg/L sodium heptanesulfonate, 34 mM K_2HPO_4, and 34 mM citric acid, pH 4.4.
Flow rate: 1.6
Detector: UV 254

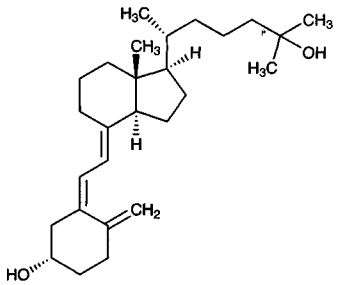
CHROMATOGRAM
Retention time: 6
Internal standard: N-ethylnornicotine (8.5)

OTHER SUBSTANCES
Extracted: metabolites, cotinine, nicotine

KEY WORDS
SPE

REFERENCE
Zuccaro,P.; Altieri,I.; Rosa,M.; Passa,A.R.; Pichini,S.; Pacifici,R. Solid-phase extraction of nicotine and its metabolites for high-performance liquid chromatographic determination in urine, *J.Chromatogr.B*, **1995**, *668*, 187–188.

Calcifediol



Molecular formula: $C_{27}H_{44}O_2$
Molecular weight: 400.65
CAS Registry No.: 19356-17-3, 63283-36-3 (monohydrate)
Merck Index: 1677
Lednicer No.: 3 101

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL MeOH, allow to stand for 30 min, add 10 mL hexane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Remove 9 mL of the organic phase and evaporate it under nitrogen at 55°, reconstitute with 300 μL mobile phase A, inject a 250 μL aliquot onto column A with mobile phase A. Collect the material corresponding to the retention time of calcifediol, evaporate it to dryness, reconstitute in mobile phase B, inject an aliquot on column B with mobile phase B.

HPLC VARIABLES

Column: A Brownlee guard column + 250 × 4.6 5 μm Li-Chrosorb SI-100; B 150 × 4.6 Ultrasphere Octyl C8
Mobile phase: A Hexane:EtOH 90:10; B MeCN:water 80:20
Flow rate: A 2; B 1.5
Injection volume: 250
Detector: UV 254

CHROMATOGRAM

Retention time: 3 (A), 4 (B)
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; normal phase

REFERENCE

Loo,J.C.; Brien,R. Analysis of 25-hydroxy vitamin D3 in plasma by high-performance liquid chromatography, *Res.Commun.Chem.Pathol.Pharmacol.*, **1983**, *41*, 139–148.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 Sep Pak SPE cartridge with 5 mL hexane, 5 mL chloroform, 5 mL MeOH, and 5 mL water. 3 mL Serum + 3 mL MeCN, vortex, centrifuge at 1500 g for 10 min. Remove the supernatant and add it to 1.5 mL 400 mM pH 10.6 K_2HPO_4, add the mixture to the SPE cartridge, wash with 5 mL water, wash with 3 mL MeOH:water 70:30, elute with 4 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb
Mobile phase: MeOH:isopropanol:hexane 2:5:93
Flow rate: 1.5
Detector: UV 254 or radioreceptor assay

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES
Extracted: calcitriol, 24,25-dihydroxyvitamin D

KEY WORDS
serum; SPE; normal phase

REFERENCE
Saggese,G.; Bertelloni,S.; Baroncelli,G.I. Dosaggio radiorecettoriale dei metaboliti della vitamina D dopo cromatografia liquida ad alta risoluzione con fasi stazionarie ultrafini, *Giorn.It.Chim.Clin.*, **1986**, *11*, 177–182.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve compound in 30 μL pyridine, add 25 μL N,O-bis-(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride, heat at 55° for 45 min, evaporate to dryness under a stream of nitrogen, reconstitute with 50 μL hexane, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 microparticulate silica
Mobile phase: Hexane:ethyl acetate 99.85:0.15
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5

KEY WORDS
derivatization; normal phase

REFERENCE
Wichmann,J.; Schnoes,H.K.; DeLuca,H.F. Isolation of identification of 24(R)-hydroxyvitamin D3 from chicks give large doses of vitamin D3, *Biochem.*, **1981**, *20*, 2350–2353.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution of calcifediol in EtOH, add 1 mL 7.2 μM DMEQ-TAD in dichloromethane, stir at room temperature for 30 min, add EtOH, evaporate, dissolve residue in MeOH, inject an aliquot. (Dichloromethane should be MeOH free. Wash with concentrated sulfuric acid, water, 5% sodium carbonate, water, dry over calcium chloride, and distil from calcium hydride. DMEQ-TAD was 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione. Synthesis is as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Shake a solution of 910 mg 4,5-dinitroveratrole in 80 mL EtOH with 89 mg platinum(IV) oxide under an atmosphere of hydrogen until the theoretical amount of hydrogen (540 mL) is absorbed, filter under nitrogen into a flask containing 580 mg 2-ketoglutaric acid, reflux this mixture for 1.5 h, cool, collect the precipitate, recrystallize from EtOH to obtain 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-propionic acid as a crystalline solid (mp 250-252°). Add a solution of 606 mg 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 20 mL DMF under nitrogen to a suspension of 176 mg NaH in 3 mL DMF stirred at 0°, stir for 30 min, add 455 μL methyl iodide, stir at 0° for 1.5 h, pour into ice-water, stir at room temperature for 30 min, acidify

with 500 mM HCl, collect the precipitate, recrystallize from chloroform/MeOH to obtain 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid (mp 239-241°). Add 360 μL triethylamine at room temperature to 500 mg 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 50 mL DMF, add 550 μL diphenylphosphoryl azide, stir at room temperature for 2.5 h, evaporate to dryness under reduced pressure, dissolve the residue in 20 mL benzene (Caution! Benzene is a carcinogen!), reflux for 1 h, cool to room temperature, add 178 mg ethyl carbazate, reflux for 30 min, evaporate, chromatograph on 90 g silica gel, elute with chloroform to remove a by-product then with chloroform:MeOH 96:4 to obtain 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]semicarbazide. Reflux a suspension of 272 mg 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]semicarbazide and 190 mg potassium carbonate in 20 mL EtOH for 6 h, evaporate the solvent, dissolve the residue in 30 mL water, acidify with 2 M HCl, extract with chloroform:MeOH 90:10, dry over anhydrous sodium sulfate, evaporate, recrystallize from MeOH/chloroform to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione as pale yellow prisms (mp 250-253°). Add 10 mg iodobenzene diacetate to a stirred suspension of 8.6 mg 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione in 1.5 mL MeOH-free dichloromethane, stir at room temperature for 3.5 h, filter, store the filtrate at -20° overnight, filter under argon to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione as red needles (mp 200-202° d).)

HPLC VARIABLES

Column: 250 × 4 LiChrospher RP-18(e)
Mobile phase: Gradient. MeOH:water from 60:40 to 80:20 over 40 min
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 33 and 36 (C6 epimers)

OTHER SUBSTANCES

Simultaneous: calcitriol

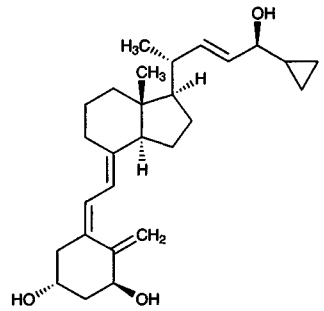
KEY WORDS

derivatization

REFERENCE

Shimizu,M.; Kamachi,S.; Nishii,Y.; Yamada,S. Synthesis of a reagent for fluorescence-labeling of vitamin D and its use in assaying vitamin D metabolites, *Anal.Biochem.*, **1991**, *194*, 77–81.

Calcipotriene



Molecular formula: $C_{27}H_{40}O_3$
Molecular weight: 412.61
CAS Registry No.: 112965-21-6
Merck Index: 1679
Lednicer No.: 5 60

SAMPLE

Matrix: cell cultures
Sample preparation: Extract cell cultures (Can. J. Biochem. 1957, 37, 911). Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 80 × 6.2 Zorbax-SIL
Mobile phase: Hexane:isopropanol:MeOH 91:7:2
Flow rate: 1
Injection volume: 200
Detector: UV 265

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

normal phase

REFERENCE

Masuda,S.; Strugnell,S.; Calverley,M.J.; Makin,H.L.J.; Kremer,R.; Jones,G. *In vitro* metabolism of the anti-psoriatic vitamin D analog, calcipotriol, in two cultured human keratinocyte models, *J.Biol.Chem.*, **1994**, *269*, 4794–4803.

SAMPLE

Matrix: cell suspensions
Sample preparation: 1.25 mL Liver homogenate + 1.25 mL MeCN, centrifuge, remove supernatant and dilute it with 2.5 mL water, pass through a Varian C8 AASP SPE cartridge, purge with 250 μL MeOH:water 10:90, elute contents of SPE cartridge onto HPLC column.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher RP 18
Mobile phase: Gradient. MeOH:water from 70:30 to 95:5 over 15 min
Flow rate: 2
Detector: UV 264

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver; human; SPE; pig

REFERENCE

Sorensen,H.; Binderup,L.; Calverley,M.J.; Hoffmeyer,L.; Andersen,N.R. *In vitro* metabolism of calcipotriol (MC 903), a vitamin D analogue, *Biochem.Pharmacol.*, **1990**, *39*, 391–393.

Calcitonin

Molecular formula: $C_{151}H_{226}N_{40}O_5S_3$
Molecular weight: 2777.9
CAS Registry No.: 9007-12-9, 47931-85-1 (salmon), 21215-62-3 (human)
Merck Index: 1680

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL rat plasma and 100 ng rat calcitonin to 1 mL polypropylene column containing immobilized antibody column (preparation details in paper), wash with three 5 mL portions of water, elute with 5 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute with MeOH:water:trifluoroacetic acid 50:50:1, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 150 × 0.32 C18 (LC Packing, Zurich)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was MeCN:water 60:40 containing 0.1% trifluoroacetic acid. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 0.003
Injection volume: 5
Detector: LC-MS, Finnigan MAT TSQ-700, Analytica model electrospray ionization (ESI) source, manifold 70°, voltage -3.5 kV, drying gas for ESI nitrogen 68 kPA at 80°, gas sheath nitrogen 102 kPa, liquid sheath 2-methoxyethanol at 2 μL/min, m/z 1140 ± 2 (human), m/z 1132 ± 2 rat

CHROMATOGRAM

Retention time: 15.3
Internal standard: rat calcitonin
Limit of quantitation: 10 ng/mL

KEY WORDS

human; rat; plasma

REFERENCE

Kobayashi,N.; Kanai,M.; Seta,K.; Nakamura,K.-i. Quantitative analysis of synthetic human calcitonin by liquid chromatography-mass spectrometry, *J.Chromatogr.B*, **1995**, *672*, 17–23.

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize (glass/PTFE homogenizer) kidney in 100 mM pH 7.4 Tris-HCl. Dilute plasma and tissue homogenates 10-fold with 0.1% trifluoroacetic acid in water, inject a 100 μL aliquot on to column a and elute to waste with mobile phase A, after 5 min backflush the contents of column A on to column B with mobile phase B and start the gradient, after 25 min re-equilibrate column A with mobile phase A and column B with mobile phase B (at initial conditions) for 5 min.

HPLC VARIABLES

Column: A 20 × 3.9 25-40 μm LiChroprep RP-8; B 10 × 4 Nova-Pak C8 + 250 × 4.6 5 μm W-Porex 5 C18 (Phenomenex)
Mobile phase: A 0.1% trifluoroacetic acid in water; B Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 75:25 to 45:55 over 20 min, to 0:100 over 5 min.
Flow rate: A 0.5; B 1.2
Injection volume: 100

Detector: radioactivity

CHROMATOGRAM
Retention time: 12
Limit of detection: 2.5 pg/mL

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
salmon; ^{125}I labeled; rat; plasma; kidney; column-switching

REFERENCE
Lee,H.S.; Lee,J.S.; Lee,H.; Jung,Y.S.; DeLuca,P.P.; Lee,K.C. Reversed-phase high-performance liquid chromatography of salmon calcitonin and its degradation products in biological samples using column switching and flow-through radioisotope detection, *J.Chromatogr.B*, **1995**, *673*, 136–141.

SAMPLE
Matrix: formulations
Sample preparation: Polymeric beads. Dissolve 100 mg dried beads in 3 mL 20 mM pH 3 glycine/HCl buffer containing 150 mM NaCl, let stand at 4° overnight, heat up to 50° for 10 min. After precipitation of the polymer, inject an aliquot of the supernatant. Release experiment. Place polymeric beads in 10 mL pH 7.4 isotonic PBS at 37° or in 10 mL 10 mM pH 4.5 acetate buffer containing 150 mM NaCl at 15°. At different times, collect 100 μL release medium and replace by the same volume of buffer. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac C4
Mobile phase: Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was MeCN:water 60:40 containing 0.095% trifluoroacetic acid. A:B from 60:40 to 40:60 over 15 min
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM
Retention time: 10

KEY WORDS
beads; stability-indicating

REFERENCE
Serres,A.; Baudys,M.; Kim,S.W. Temperature and pH-sensitive polymers for human calcitonin delivery, *Pharm.Res.*, **1996**, *13*, 196–201.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 4.6 5 μm M-C4 (pore diameter 60 Å) (ES Industries)
Mobile phase: Gradient. A was 10 mM ammonium acetate containing 0.1% trifluoroacetic acid. B was MeCN:20 mM ammonium acetate 50:50 containing 0.1% trifluoroacetic acid. A:B 45:5 for 5 min, to 40:60 over 35 min, maintain at 40:60 over 5 min.
Flow rate: 2
Injection volume: 200
Detector: UV 214

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
salmon

REFERENCE
Lee,I.H.; Pollack,S.; Hsu,S.H.; Miksic,J.R. Influence of the mobile phase on salmon calcitonin analysis by reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1991**, *29*, 136–140.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in buffer, inject a 100 μL aliquot. (Buffer was 2 mg/mL sodium acetate and 5 mg/mL NaCl adjusted to pH 4.2 with glacial acetic acid.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm M-C4 (pore diameter 60 Å) (ES Industries)
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 27.5:72.5 to 30:70 over 15 min
Flow rate: 2
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 3 (human), 7.5 (salmon), 9 (eel), 13.5 (pig)

KEY WORDS
salmon; human; eel; pig

REFERENCE
Lee,I.H.; Pollack,S.; Hsu,S.H.; Miksic,J.R. Influence of the mobile phase on salmon calcitonin analysis by reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1991**, *29*, 136–140.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, gonadorelin (LH-RH), protirelin (TRH), somatostatin

KEY WORDS
human

REFERENCE

Varian Catalog, **1993**, p. 182.

SAMPLE

Matrix: solutions

Sample preparation: 5 μL Calcitonin in MeCN:0.2% aqueous phosphoric acid containing 100 mM sodium perchlorate 30:70 + 20 μL 250 mM pH 8.5 borate buffer containing 2.5 mM sodium dodecyl sulfate + 2.5 μL 30 mM 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in MeCN, add 11 μL MeCN and 11.5 μL water, heat at 50 ° for 3 h, cool, add 5 μL 1 M HCl, add 40 μL MeCN, add 5 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS 80Tm (Tosoh)

Mobile phase: Gradient. A was MeCN containing 0.05% trifluoroacetic acid. B was water containing 0.05% trifluoroacetic acid. A:B 33:67 for 8 min, to 67:33 over 17 min.

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: F ex 430 em 558

CHROMATOGRAM

Retention time: 26

Limit of detection: 71 pg

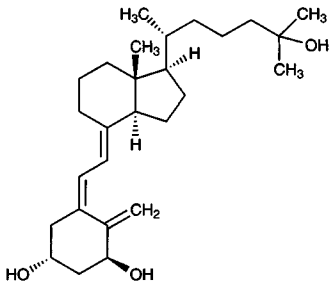
KEY WORDS

salmon; derivatization

REFERENCE

Fukuda,T.; Ishikawa,K.; Imai,K. Sensitive determination of salmon calcitonin, by means of pre-column derivatization, HPLC and fluorometric determination, *Biomed.Chromatogr.*, **1995**, *9*, 52–55.

Calcitriol



Molecular formula: $C_{27}H_{44}O_3$
Molecular weight: 416.64
CAS Registry No.: 32222-06-3
Merck Index: 1681
Lednicer No.: 3 103

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL MeOH, allow to stand for 30 min, add 10 mL hexane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Remove 9 mL of the organic phase and evaporate it under nitrogen at 55°, reconstitute with 300 μL mobile phase, inject a 250 μL aliquot.

HPLC VARIABLES

Guard column: Brownlee guard column
Column: 250 × 4.6 5 μm Li-Chrosorb SI-100
Mobile phase: Hexane:EtOH 90:10
Flow rate: 2
Injection volume: 250
Detector: UV 254

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: calcifediol

KEY WORDS

plasma; normal phase

REFERENCE

Loo,J.C.; Brien,R. Analysis of 25-hydroxy vitamin D3 in plasma by high-performance liquid chromatography, *Res.Commun.Chem.Pathol.Pharmacol.*, **1983**, *41*, 139–148.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 Sep Pak SPE cartridge with 5 mL hexane, 5 mL chloroform, 5 mL MeOH, and 5 mL water. 3 mL Serum + 3 mL MeCN, vortex, centrifuge at 1500 g for 10 min. Remove the supernatant and add it to 1.5 mL 400 mM pH 10.6 K_2HPO_4, add the mixture to the SPE cartridge, wash with 5 mL water, wash with 3 mL MeOH:water 70:30, elute with 4 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb
Mobile phase: MeOH:isopropanol:hexane 2:5:93
Flow rate: 1.5
Detector: UV 254 or radioreceptor assay

CHROMATOGRAM

Retention time: 9.25

OTHER SUBSTANCES
Extracted: calcifediol (25-hydroxyvitamin D3), 24,25-dihydroxyvitamin D

KEY WORDS
serum; SPE; normal phase

REFERENCE
Saggese,G.; Bertelloni,S.; Baroncelli,G.I. Dosaggio radiorecettoriale dei metaboliti della vitamina D dopo cromatografia liquida ad alta risoluzione con fasi stazionarie ultrafini, *Giorn.It.Chim.Clin.*, **1986**, *11*, 177–182.

SAMPLE
Matrix: formulations
Sample preparation: Cut 25 capsules in half and add to 5 mL isooctane:isopropanol 90:10, filter through glass wool, wash capsule shells with three 5 mL aliquots of isooctane:isopropanol 90:10, combine filtrates and make up to 25 mL with isooctane:isopropanol 90:10, inject a 50 μL aliquot. Alternatively, inject directly 10 μL aliquots of oil in capsules.

HPLC VARIABLES
Column: Two 150 × 4.6 5 μm Ultrasphere-SI silica in series
Mobile phase: Hexane:THF:dichloromethane:isopropanol 72:12:12:4
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 16.3

OTHER SUBSTANCES
Simultaneous: 1α-hydroxycholecalciferol (alfacalcidol)

KEY WORDS
capsules; normal phase

REFERENCE
Flann,B.C.; Lodge,B.A. Validation of liquid chromatographic method for assay of calcitriol and alfacalcidol in capsule formulations, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 1026–1030.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution of calcifediol in EtOH, add 1 mL 7.2 μM DMEQ-TAD in dichloromethane, stir at room temperature for 30 min, add EtOH, evaporate, dissolve residue in MeOH, inject an aliquot. (Dichloromethane should be MeOH free. Wash with concentrated sulfuric acid, water, 5% sodium carbonate, water, dry over calcium chloride, and distil from calcium hydride. DMEQ-TAD was 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione. Synthesis is as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Shake a solution of 910 mg 4,5-dinitroveratrole in 80 mL EtOH with 89 mg platinum(IV) oxide under an atmosphere of hydrogen until the theoretical amount of hydrogen (540 mL) is absorbed, filter under nitrogen into a flask containing 580 mg 2-ketoglutaric acid, reflux this mixture for 1.5 h, cool, collect the precipitate, recrystallize from EtOH to obtain 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-

propionic acid as a crystalline solid (mp 250-252°). Add a solution of 606 mg 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 20 mL DMF under nitrogen to a suspension of 176 mg NaH in 3 mL DMF stirred at 0°, stir for 30 min, add 455 μL methyl iodide, stir at 0° for 1.5 h, pour into ice-water, stir at room temperature for 30 min, acidify with 500 mM HCl, collect the precipitate, recrystallize from chloroform/MeOH to obtain 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid (mp 239-241°). Add 360 μL triethylamine at room temperature to 500 mg 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 50 mL DMF, add 550 μL diphenylphosphoryl azide, stir at room temperature for 2.5 h, evaporate to dryness under reduced pressure, dissolve the residue in 20 mL benzene (Caution! Benzene is a carcinogen!), reflux for 1 h, cool to room temperature, add 178 mg ethyl carbazate, reflux for 30 min, evaporate, chromatograph on 90 g silica gel, elute with chloroform to remove a by-product then with chloroform:MeOH 96:4 to obtain 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)]ethylsemicarbazide. Reflux a suspension of 272 mg 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]semicarbazide and 190 mg potassium carbonate in 20 mL EtOH for 6 h, evaporate the solvent, dissolve the residue in 30 mL water, acidify with 2 M HCl, extract with chloroform:MeOH 90:10, dry over anhydrous sodium sulfate, evaporate, recrystallize from MeOH/chloroform to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione as pale yellow prisms (mp 250-253°). Add 10 mg iodobenzene diacetate to a stirred suspension of 8.6 mg 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione in 1.5 mL MeOH-free dichloromethane, stir at room temperature for 3.5 h, filter, store the filtrate at -20° overnight, filter under argon to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione as red needles (mp 200-202° d).)

HPLC VARIABLES

Column: 250 × 4 LiChrospher RP-18(e)
Mobile phase: Gradient. MeOH:water from 60:40 to 80:20 over 40 min
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 27 and 30 (C6 epimers)

OTHER SUBSTANCES

Simultaneous: calcifediol

KEY WORDS

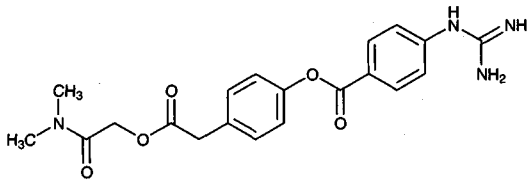
derivatization

REFERENCE

Shimizu,M.; Kamachi,S.; Nishii,Y.; Yamada,S. Synthesis of a reagent for fluorescence-labeling of vitamin D and its use in assaying vitamin D metabolites, *Anal.Biochem.*, **1991**, *194*, 77–81.

Camostat

Molecular formula: $C_{20}H_{22}N_4O_5$
Molecular weight: 398.42
CAS Registry No.: 59721-28-7
Merck Index: 1775



SAMPLE

Matrix: bile, duodenal fluid, pancreatic juice
Sample preparation: 500 µL Bile, pancreatic juice, or duodenal juice + 5 µL 100 µg/mL 4-guanidinobenzanilide methanesulfonate in water + 100 µL 2 M methanesulfonate, purify using a Sep-Pak C18 SPE cartridge.

HPLC VARIABLES

Column: 150 × 4.6 YMC-Pack A302 ODS (Shimakyu)
Mobile phase: MeOH:water:acetic acid:5% sodium dodecyl sulfate in 50% EtOH:5% sodium heptanoyl sulfate in 50% EtOH 200:140:1:1.5:4
Flow rate: 0.8
Detector: UV 265

OTHER SUBSTANCES

Extracted: metabolites

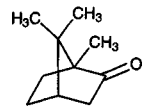
KEY WORDS

dog; SPE

REFERENCE

Kitagawa,M.; Hayakawa,T.; Kondo,T.; Shibata,T.; Sugimoto,Y. Pancreatic and biliary excretion of camostat in dogs, *Digestion*, **1988**, *39*, 204–209.

Camphor



Molecular formula: $C_{10}H_{16}O$
Molecular weight: 152.24
CAS Registry No.: 76-22-2
Merck Index: 1779

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 5 mL Plasma + 10 mL peroxide-free ether, mix. Remove the organic layer and add it to 2 mL isopropanol, evaporate the mixture to 2 mL under a stream of nitrogen at room temperature, add 2 mL of a saturated solution of 2,4-dinitrophenylhydrazine in MeOH, add 1 drop of concentrated HCl, heat at 85-90° for 18 h, cool, evaporate under a stream of nitrogen at 50° to 1 mL, add 2 mL 2 M HCl, evaporate to 2 mL, add 2 mL 2 M HCl, add 4 mL isooctane, rotate mechanically for 30 min. Remove the organic layer and wash it twice with 4 mL water, centrifuge, dry over anhydrous sodium sulfate, add to a florisil column, elute with 10 mL alcohol-free chloroform, evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 2 mL MeCN:water 70:30, inject a 200 μL aliquot. Urine. Adjust 40 mL urine to pH 6.5 with three drops 6 M HCl, saturate with NaCl, extract with 10 mL peroxide-free ether. Remove the organic layer and add it to 2 mL isopropanol, evaporate the mixture to 2 mL under a stream of nitrogen at room temperature, add 2 mL of a saturated solution of 2,4-dinitrophenylhydrazine in MeOH, add 1 drop of concentrated HCl, heat at 85-90° for 18 h, cool, evaporate under a stream of nitrogen at 50° to 1 mL, add 2 mL 2 M HCl, evaporate to 2 mL, add 2 mL 2 M HCl, add 4 mL isooctane, rotate mechanically for 30 min. Remove the organic layer and wash it twice with 4 mL water, centrifuge, dry over anhydrous sodium sulfate, add to a florisil column, elute with 10 mL alcohol-free chloroform, evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 2 mL MeCN:water 70:30, inject a 50 μL aliquot. (The florisil (60-100 mesh; J.T. Baker) was heated at 120° overnight, cooled, and 3% water added. The column was prepared by plugging the end of a Pasteur pipette with glass wool and adding florisil to a height of 6 cm followed by anhydrous sodium sulfate to a height of 1 cm, pre-wash with 3 mL isooctane.)

HPLC VARIABLES

Column: radial compression μBondapak C18
Mobile phase: MeCN:water 82:18
Flow rate: 1
Injection volume: 50-200
Detector: UV 368.5

CHROMATOGRAM

Retention time: 14.5
Limit of detection: 20 ng/mL (plasma), 10 ng/mL (urine)

KEY WORDS

plasma; horse; derivatization; SPE

REFERENCE

Gallicano,K.D.; Park,H.C.; Young,L.M. A sensitive liquid chromatographic procedure for the analysis of camphor in equine urine and plasma, *J.Anal.Toxicol.*, **1985**, *9*, 24–30.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 1 5 μm LiChrosorb RP18

Mobile phase: EtOH:water 35:65 containing 20 mM α-cyclodextrin and 0.5 mM tri-O-methyl-α-cyclodextrin
Column temperature: 25
Flow rate: 0.04
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: k' 7.9, k' 9.3 (enantiomers)

OTHER SUBSTANCES

Extracted: fenchone

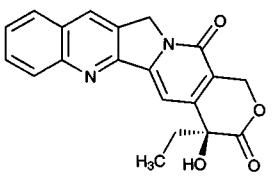
KEY WORDS

chiral

REFERENCE

Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

Camptothecin



Molecular formula: $C_{20}H_{16}N_2O_4$
Molecular weight: 348.36
CAS Registry No.: 7689-03-4
Merck Index: 1783

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge (Waters) with 1 mL MeOH and 1 mL water. Vortex 100 μL plasma and 10 μL 20 nM IS in water for 5 s, add to the SPE cartridge, wash twice with 1 mL water, wash with 1 mL MeOH:water 20:80. Elute with 1 mL MeOH:25 mM pH 2.55 KH_2PO_4 75:25, store at -70°, thaw, inject an aliquot.

HPLC VARIABLES

Guard column: μBondapak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM pH 4.8 KH_2PO_4 containing 1 mM sodium heptanesulfonate 35:65
Flow rate: 0.85
Detector: F ex 360 em 440

CHROMATOGRAM

Retention time: 6.0
Internal standard: 7-ethyl-10-hydroxycamptothecin (4.7)
Limit of quantitation: 5.74 nM

KEY WORDS

SPE; pharmacokinetics; plasma; procedure measures only lactone form of camptothecin

REFERENCE

Ahmed,F.; Vyas,V.; Saleem,A.; Li,X.-G.; Zamek,R.; Cornfield,A.; Haluska,P.; Ibrahim,N.; Rubin,E.H.; Gupta,E. High-performance liquid chromatographic quantitation of total and lactone 20(S)camptothecin in patients receiving oral 20(S)camptothecin, *J.Chromatogr.B*, **1998**, *707*, 227–233.

SAMPLE

Matrix: blood
Sample preparation: Vortex 100 μL plasma, 10 μL 20 nM IS in water, and 300 μL MeOH for 5 s, centrifuge at 5800 g for 3 min. Acidify the clear supernatant with 50 μL 500 mM hydrochloric acid, inject an aliquot.

HPLC VARIABLES

Guard column: μBondapak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM pH 4.8 KH_2PO_4 containing 1 mM sodium heptanesulfonate 35:65
Flow rate: 0.85
Detector: F ex 360 em 440

CHROMATOGRAM

Retention time: 6.0
Internal standard: 7-ethyl-10-hydroxycamptothecin (4.7)
Limit of quantitation: 5.74 nM

KEY WORDS

pharmacokinetics; plasma; procedure measures total camptothecin (carboxylate and lactone)

REFERENCE

Ahmed,F.; Vyas,V.; Saleem,A.; Li,X.-G.; Zamek,R.; Cornfield,A.; Haluska,P.; Ibrahim,N.; Rubin,E.H.; Gupta,E. High-performance liquid chromatographic quantitation of total and lactone 20(S)camptothecin in patients receiving oral 20(S)camptothecin, *J.Chromatogr.B*, **1998**, *707*, 227–233.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL MeOH:10 mM HCl 40:60 + 800 mg solid NaCl, extract with 7.5 mL MeCN:n-butyl chloride 20:80 for 5 min, centrifuge at 4000 g for 2 min. Rotate quickly by hand to break the gels, centrifuge at 4000 g for 5 min. Mix the organic layer with 50 μL DMSO, dry under a gentle stream of nitrogen at 60° to approximately 50 μL. Reconstitute the residue in 100 μL MeOH and 100 μL perchloric acid:water 1:500, vortex for 5 s, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18

Column: 100 × 4.6 5 μm Hypersil ODS

Mobile phase: MeOH:buffer 40:60 adjusted to pH 5.5 with HCl (Buffer was 100 mM ammonium acetate containing 10 mM tetrabutylammonium sulfate.)

Column temperature: 50

Flow rate: 1

Injection volume: 100

Detector: F ex 355 em 515

CHROMATOGRAM

Retention time: 6.5

Internal standard: camptothecin

OTHER SUBSTANCES

Extracted: irinotecan

Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, metoclopramide, morphine, ranitidine

KEY WORDS

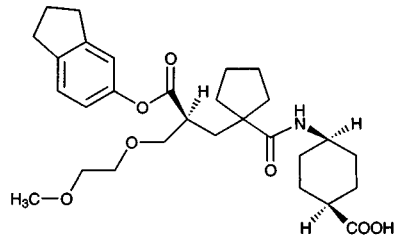
plasma; camptothecin is IS

REFERENCE

de Bruijn,P.; Verweij,J.; Loos,W.J.; Nooter,K.; Stoter,G.; Sparreboom,A. Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *698*, 277–285.

Candoxatril

Molecular formula: $C_{29}H_{41}NO_7$
Molecular weight: 515.65
CAS Registry No.: 123122-55-4



SAMPLE
Matrix: blood
Sample preparation: Add 100 µL 2 M HCl to 1 mL plasma, extract with MTBE containing IS. Evaporate the organic phase, reconstitute the residue in 150 µL MeOH:2 mM ammonium acetate 50:50. Inject a 100 µL aliquot.

HPLC VARIABLES
Column: 30 × 4.6 C8
Mobile phase: MeOH:2 mM ammonium acetate 50:50
Flow rate: 1.0
Injection volume: 100
Detector: MS; PE SCIEX API III plus negative ion mode, m/z 398

CHROMATOGRAM
Retention time: 1.5
Internal standard: UK 77568 (Pfizer) (1.1, m/z 378)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: candoxatrilat (active metabolite, 0.8, m/z 398)

KEY WORDS
plasma; mouse; rabbit; human

REFERENCE
Kaye,B.; Brearley,C.J.; Cussans,N.J.; Herron,M.; Humphrey,M.J.; Mollatt,A.R. Formation and pharmacokinetics of the active drug candoxatrilat in mouse, rat, rabbit, dog and man following administration of the prodrug candoxatril, *Xenobiotica*, **1997**, *27*, 1091–1102.

SAMPLE
Matrix: urine
Sample preparation: Inject a 500 µL aliquot directly.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Inertsil ODS-2 (GL Sciences Inc.)
Mobile phase: Gradient. A was MeOH. B was 20 mM ammonium acetate. A:B from 0:100 to 100:0 over 20 min, maintain at 100:0 for 10 min.
Injection volume: 500
Detector: Radioactivity, Ramona-5 flow detector

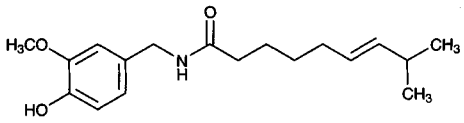
CHROMATOGRAM
Internal standard: UK 77568 (Pfizer)

OTHER SUBSTANCES
Extracted: candoxatrilat (active metabolite)

KEY WORDS
radiolabeled

REFERENCE
Kaye,B.; Brearley,C.J.; Cussans,N.J.; Herron,M.; Humphrey,M.J.; Mollatt,A.R. Formation and pharmacokinetics of the active drug candoxatrilat in mouse, rat, rabbit, dog and man following administration of the prodrug candoxatril, *Xenobiotica*, **1997**, *27*, 1091–1102.

Capsaicin



Molecular formula: $C_{18}H_{27}NO_3$
Molecular weight: 305.42
CAS Registry No.: 404-86-4
Merck Index: 1811

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 300 μL acetone + 20 ng dihydrocapsaicin, homogenize, centrifuge at 13000 g for 2 min. Filter the supernatant (0.45 μm), inject a 5-20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5Ph (Nakarai Chemicals)
Mobile phase: MeCN:100 mM KH_2PO_4 45:55, pH 5.0
Flow rate: 1
Injection volume: 5-20
Detector: E, Irica E-502, glassy carbon electrode + 750 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.5
Internal standard: dihydrocapsaicin (10)
Limit of detection: 12 pg

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Kawada,T.; Watanabe,T.; Katsura,K.; Takami,H.; Iwai,K. Formation and metabolism of pungent principle of *Capsicum* fruits. XV. Microdetermination of capsaicin by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *329*, 99–105.

SAMPLE

Matrix: bulk
Sample preparation: Grind to pass 200 Mesh screen. Reflux 10 g in 100 mL acetone, cool, filter (Whatman No. 41 filter paper), evaporate the filtrate under reduced pressure. Dissolve the residue in 50 mL denatured EtOH, dilute a 5 mL aliquot to 10 mL with MeOH: water 50:50, filter (0.5 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm C8 (Supelco)
Mobile phase: Dissolve 1.85 g sodium pentanesulfonate in 400 mL water, make up to 1 L with MeOH.
Flow rate: 2
Injection volume: 20
Detector: UV 200

CHROMATOGRAM

Retention time: 6.5

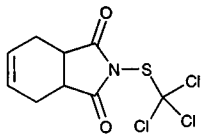
OTHER SUBSTANCES

Simultaneous: nordihydrocapsaicin, dihydrocapsaicin

REFERENCE

Weaver,K.M.; Awde,D.B. Rapid high-performance liquid chromatographic method for the determination of very low capsaicin levels, *J.Chromatogr.*, **1986**, *367*, 438–442.

Captan



Molecular formula: $C_9H_8Cl_3NO_2S$
Molecular weight: 300.59
CAS Registry No.: 133-06-2
Merck Index: 1815

SAMPLE

Matrix: formulations
Sample preparation: Add a 200 mg tablet containing 6 mg captan and 5 mg sulfur to 5 mL carbon disulfide, extract the solid residue five times with 5 mL carbon disulfide, combine the extracts and evaporate to constant weight. Dissolve the residue in 2 mL carbon disulfide, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Perkin-Elmer C8
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 3.2

OTHER SUBSTANCES

Simultaneous: sulfur

KEY WORDS

tablets

REFERENCE

Fedeli,G.; Moltrasio,D.; Aleotti,M.; Gazzani,G. High-performance liquid chromatographic determination of sulphur and captan in a mixture, *J.Chromatogr.*, **1988**, *447*, 263–267.

SAMPLE

Matrix: solutions
Sample preparation: Pass 100 mL water through column A at 5 mL/min then elute the contents of column A onto column B with the mobile phase, elute column B with the mobile phase and monitor the effluent fom column B.

HPLC VARIABLES

Column: A 30 × 4.6 5 μm Spherrisorb ODS C18; B 250 × 4.6 5 μm Supelcosil LC-8 C8
Mobile phase: Gradient. MeCN:water 30:70 for 5 min, to 60:40 over 10 min, maintain at 60:40 for 10 min, to 30:70 over 5 min, maintain at 30:70 for 5 min and inject next sample.
Flow rate: 1.5
Injection volume: 100000
Detector: UV 220

CHROMATOGRAM

Retention time: 21.10
Limit of detection: 460 pg/mL

OTHER SUBSTANCES

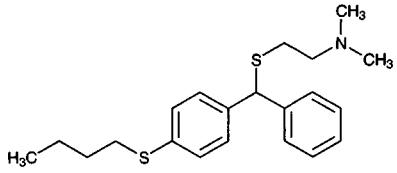
Simultaneous: propoxur, carbofuran, carbaryl, propham, chloropropham, barban, butylate

KEY WORDS
water; drinking water; column-switching

REFERENCE

Marvin,C.H.; Brindle,I.D.; Hall,C.D.; Chiba,M. Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water, *J.Chromatogr.*, **1990**, *503*, 167–176.

Captodiamine



Molecular formula: $C_{21}H_{29}NS_2$
Molecular weight: 359.60
CAS Registry No.: 486-17-9, 904-04-1 (HCl)
Merck Index: 1816
Lednicer No.: 1 44

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 20.15

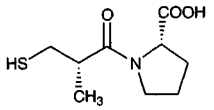
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Captopril



Molecular formula: $C_9H_{15}NO_3S$
Molecular weight: 217.29
CAS Registry No.: 62571-86-2
Merck Index: 1817
Lednicer No.: 3 128; 4 7, 58, 81, 128

SAMPLE

Matrix: blood
Sample preparation: Mix 100 μL plasma with 100 μL 100 mM pH 7.5 borate buffer and 10 μL 10% monobromobimane in MeCN, vortex for 15 s, let stand at room temperature for 5 min, add 200 μL MeCN, vortex, centrifuge at 3000 g for 5 min. Keep the supernatant at -20° prior to analysis. Inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C 18 Guard-Pak
Column: 150 × 3.9 4 μm NovaPak C 18
Mobile phase: MeCN:water:trifluoroacetic acid 20:80:0.1
Column temperature: 50
Flow rate: 1.0
Injection volume: 10
Detector: F ex 400 em 480

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 30 pg
Limit of quantitation: 12.5 ng/mL

KEY WORDS

plasma; derivatization; pharmacokinetics; human; rat

REFERENCE

Kok,R.J.; Visser,J.; Moolenaar,F.; De Zeeuw,D.; Meijer,D.K.F. Bioanalysis of captopril: two sensitive high-performance liquid chromatographic methods with pre-or postcolumn fluorescent labeling, *J.Chromatogr.B*, **1997**, *693*, 181–189.

SAMPLE

Matrix: blood
Sample preparation: Add 30 μL 1 mg/mL p-bromophenacyl bromide in MeOH and 20 μL 50 μg/mL IS in MeOH to 500 μL plasma. Mix for 30 s and let stand at room temperature for 20 min. Add 100 μL 1 M HCL, mix for 15-20 s. Extract with 4 mL ethyl acetate: benzene 50:50 (Caution! Benzene is a carcinogen!). Mix for 30 s and then shake gently for 5 min. Centrifuge and remove the organic layer, evaporate it to dryness, reconstitute the residue in 200 μL MeCN. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 30 × 0.4 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 52.5:47.1:0.4
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 4.39

Internal standard: captopril-DDPM adduct (Prepare captopril-DDPM adduct as follows. Weigh 150 mg captopril and 250 mg N-(4-dimethylamino-3,5-dinitrophenyl)maleimide (DDPM), add 40 ml 20 mM pH 7.0 phosphate buffer and 30 mL acetone. Heat in a water-bath for 15 min and let stand at room temperature overnight. Evaporate to dryness under vacuum and add 100 mL water. Make alkaline with 2 M NaOH (the color changes from orange to deep red) and wash 4 times with 25 mL portions of ethyl acetate. Acidify the aqueous layer with 1 M HCl and extract twice with 25 mL portions of ethyl acetate. Dry the organic layer over magnesium sulfate and evaporate to yield 300 mg captopril-DDPM adduct as a deep red oil. Dissolve in 10-15 mL ethyl acetate and precipitate with 100 mL hexane.) (3.62)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Klein,J.; Colin,P.; Scherer,E.; Levy,M.; Koren,G. Simple measurement of captopril in plasma by high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1990**, *12*, 105–110.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bakerbond C18 SPE cartridge with two 200 μL portions of MeOH and two 200 μL portions of water. 1 mL Plasma + 100 μL 200 mM disodium EDTA + 100 μL 200 mM ascorbic acid + 200 μL 1 μg/mL IS + 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Combine the supernatant and the washings and neutralize (indicator paper) them with 1 M NaOH, add 3 mL 1 M pH 8.2 Tris buffer, add 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, let stand for 15 min, adjust pH to 2.5-3.0 (indicator paper) with 4 M phosphoric acid, centrifuge, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, wash with three 100 μL portions of MeCN, dry under vacuum for 5 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot. To measure total captopril proceed as follows. 1 mL Plasma + 100 μL 200 mM disodium EDTA + 100 μL 200 mM ascorbic acid + 200 μL 1 μg/mL IS + 2 mL 100 mM perchloric acid + 100 μL 40 mg/mL triphenylphosphine in MeCN, mix, heat at 50° for 40 min, cool, add 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Combine the supernatant and the washings and neutralize (indicator paper) them with 1 M NaOH, add 3 mL 1 M pH 8.2 Tris buffer, add 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, let stand for 15 min, adjust pH to 2.5-3.0 (indicator paper) with 4 M phosphoric acid, centrifuge, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, wash with three 100 μL portions of MeCN, dry under vacuum for 5 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot. To measure protein-conjugated captopril proceed as follows. 1 mL Plasma + 100 μL 200 mM disodium EDTA + 100 μL 200 mM ascorbic acid + 200 μL 1 μg/mL IS + 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Suspend the precipitate in 2 mL 100 mM perchloric acid, add 200 μL 1 μg/mL IS, add 100 μL 40 mg/mL triphenylphosphine in MeCN, heat at 50° with occasional shaking for 40 min, cool, add 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Combine the supernatant and the washings and neutralize (indicator paper) them with 1 M NaOH, add 3 mL 1 M pH 8.2 Tris buffer, add 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, let stand for 15 min, adjust pH to 2.5-3.0 (indicator paper) with 4 M phosphoric acid, centrifuge, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, wash with three 100 μL portions of MeCN, dry under vacuum for 5 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:

water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot. (Prepare 1-benzyl-2-chloropyridinium bromide as follows. Add 8.5 g benzyl bromide to 4.5 g 2-chloropyridine with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloropyridinium bromide (mp 187-191°).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil
Column: 100 × 2.1 5 μm ODS Hypersil
Mobile phase: Acetone:buffer 25:75 (Buffer was 200 mM pH 2.5 citrate buffer containing 10 mM sodium octanesulfonate and 15 mM NaCl.)
Column temperature: 50
Flow rate: 0.2
Injection volume: 20
Detector: UV 314

CHROMATOGRAM
Retention time: 10
Internal standard: 1-benzyl-2-chloro-4-methylpyridinium bromide-captopril adduct (Preparation is as follows. Add 8.5 g benzyl bromide to 5.1 g 2-chloro-4-methylpyridine (Loba-Chemie, Vienna) with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloro-4-methylpyridinium bromide. Condition a 1 g C18 SPE cartridge with two 1 mL portions of MeOH and with 1 mL water. Stir 30 mg captopril and 80 mg 1-benzyl-2-chloro-4-methylpyridinium bromide in 5 mL 500 mM pH 8.2 Tris buffer for 1 h, add 1.6 mL 100 mM sodium sulfide, let stand for 10 min, add to the SPE cartridge, wash with two 1 mL portions of water, dry under vacuum for 10 min, wash with three 1 mL portions of MeCN, dry under vacuum for 5 min, elute with 5 mL MeOH:water 80:20. Dilute the eluate to 10 mL with water, dilute with water to an adduct concentration of 3 μg/mL. 2-Chloro-4-methylpyridine can also be prepared as follows. Stir 3.6 g 2-amino-4-picoline (2-amino-4-methylpyridine) in 75 g concentrated HCl at between -15° and -20°, saturate with hydrogen chloride gas, add 3.5 g pulverized sodium nitrite in small portions, let stand overnight, neutralize, steam distil to give 2-chloro-4-methylpyridine as a colorless liquid (bp 194-195°) (Ber. 1924, 57, 791).) (12)
Limit of quantitation: 10 ng/mL

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Bald,E.; Sypniewski,S.; Drzewoski,J.; Stepien,M. Application of 2-halopyridinium salts as ultraviolet derivatization reagents and solid-phase extraction for determination of captopril in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 283–289.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 1 mg/mL p-bromophenacyl bromide in MeOH + 20 μL 50 μg/mL 4-chloro-2-nitroaniline in MeOH, vortex for 30 s, let stand at room temperature for 15 min, add 200 μL 2 M HCl, vortex for 10 s, add 5 mL benzene:ethyl acetate 50:50 (Caution! Benzene is a carcinogen!), vortex for 2 min. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeCN, vortex for 20 s, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb C18
Mobile phase: MeCN:water:acetic acid 44:55:0.2
Flow rate: 1.4
Injection volume: 25
Detector: UV 258

CHROMATOGRAM
Retention time: 3.6
Internal standard: 4-chloro-2-nitroaniline (4.7)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Li,K.; Tan,L.; Zhou,J.A. HPLC determination of captopril in human plasma and its pharmacokinetic study, *Biomed.Chromatogr.*, **1996**, *10*, 237–239.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL silica SPE cartridge with 1 mL benzene (Caution! Benzene is a carcinogen!). Collect 10 mL blood in a tube containing 30 mg 2,4'-dibromoacetophenone (p-bromophenacyl bromide), vortex for 30 s, let stand at room temperature for at least 15 min. Remove the serum and freeze it at -20°. Thaw serum and add 1 mL to 200 μL 1 M HCl, mix, extract twice with 2 mL portions of benzene. Add the organic layers to the SPE cartridge, wash with 4 mL benzene, elute with 500 μL MeCN, add 50 μL 10 μg/mL IS in acetone to the eluate, make up to 1 mL with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Spheri-5 ODS-GU
Column: 100 × 4.6 5 μm Spheri-5 ODS
Mobile phase: MeCN:water:acetic acid 45:54:1
Flow rate: 1
Injection volume: 100
Detector: UV 263

CHROMATOGRAM
Retention time: 4
Internal standard: thiosalicylic acid-p-bromophenacyl bromide adduct (Prepare by dissolving 2.4 mmoles thiosalicylic acid and 2.4 mmoles p-bromophenacyl bromide in 40 mL MeOH, adjust to pH 7 by the dropwise addition of 1 M NaOH, allow to stand at room temperature for 10 min, evaporate to dryness under reduced pressure, reconstitute with 40 mL 50 mM pH 7.0 phosphate buffer, wash twice with 20 mL portions of hexane, adjust pH to 2 with dilute HCl, extract with 40 mL ethyl acetate, evaporate to dryness under reduced pressure, recrystallize the residue from benzene to give the adduct as pale yellow plates (Chem. Pharm. Bull. 1981, 29, 150).) (8)
Limit of detection: 15 ng/mL

KEY WORDS
derivatization; SPE; whole blood; pharmacokinetics

REFERENCE
Bahmaei,M.; Khosravi,A.; Zamiri,C.; Massoumi,A.; Mahmoudian,M. Determination of captopril in human serum by high performance liquid chromatography using solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1181–1186.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Free captopril determination. Mix 100 μL plasma with 100 μL 100 mM pH 7.5 borate buffer and 300 μL MeOH, vortex, centrifuge at 3000 g for 5 min, inject a 25 μL aliquot directly. Plasma, urine. Total captopril determination. Mix

100 μL plasma or diluted urine (1:10) with 100 μL 100 mM pH 7.5 borate buffer and 100 μL 1% tributylphosphine in MeOH, vortex for 15 s, allow to stand at room temperature for 15 min, add 200 μL MeOH, vortex, centrifuge at 3000 g for 5 min. Let the supernatant stand at room temperature for at least 1 h. Inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C 18 Guard-Pak
Column: 150 × 3.9 4 μm NovaPak C 18
Mobile phase: MeCN:water:trifluoroacetic acid 15:85:0.1 containing 100 mg/L glycine
Column temperature: 50
Flow rate: 0.8
Injection volume: 25
Detector: F ex 345 em 455 following post-column reaction. The column effluent mixed with 100 mM pH 8.5 borate buffer containing 100 mg/L o-phthalaldehyde pumped at 1.2 mL/min and this mixture flowed through a 1 m × 0.5 mm I.D. mixing coil to the detector.

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 50 pg (plasma)
Limit of quantitation: 25 ng/mL (plasma), 250 ng/mL (urine)

KEY WORDS
plasma; post-column reaction; pharmacokinetics; human; rat

REFERENCE
Kok,R.J.; Visser,J.; Moolenaar,F.; De Zeeuw,D.; Meijer,D.K.F. Bioanalysis of captopril: two sensitive high-performance liquid chromatographic methods with pre-or postcolumn fluorescent labeling, *J.Chromatogr.B*, **1997**, *693*, 181–189.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Bakerbond C18 SPE cartridge with two 200 μL portions of MeOH and two 200 μL portions of water. Whole blood. 3 mL Whole blood + 100 μL 100 mM EDTA + 100 μL 200 mM ascorbic acid + 2 mL 1 M pH 8.2 Tris buffer + 200 μL 3 μg/mL IS + 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, vortex for 15 min, centrifuge at 3000 g for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 400 μL 3 M perchloric acid, centrifuge for 15 min, rinse the precipitate with 500 μL portions of water. Combine the supernatant and the rinses and adjust the pH to 2.5-3.0 (indicator paper) with 100 mM NaOH, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL water, inject a 20 μL aliquot. Urine. 500 μL Urine + 100 μL 200 mM EDTA + 100 μL 200 mM ascorbic acid + 3 mL 1 M pH 8.2 Tris buffer + 200 μL 3 μg/mL IS + 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, vortex for 15 min, adjust the pH to 2.5-3.0 with 4 M phosphoric acid, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL water, inject a 20 μL aliquot. (Prepare 1-benzyl-2-chloropyridinium bromide as follows. Add 8.5 g benzyl bromide to 4.5 g 2-chloropyridine with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloropyridinium bromide (mp 187-191°) (J. Chromatogr. B 1996, 681, 283).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil
Column: 150 × 3.3 7 μm Separon SGX (Struzeni, Prague)
Mobile phase: Gradient. A was MeCN:100 mM pH 2.5 citric acid buffer containing 20 mM sodium octanesulfonate 25:75. B was MeCN:MeOH 50:50. A:B 100:0 for 10 min, to 80:20

over 10 min, maintain at 80:20 for 5 min, to 60:40 over 5 min, return to initial conditions over 7 min.
Column temperature: 50
Flow rate: 0.5
Injection volume: 20
Detector: UV 314

CHROMATOGRAM

Retention time: 23
Internal standard: 1-benzyl-2-chloro-4-methylpyridinium bromide-captopril adduct (Preparation is as follows. Add 8.5 g benzyl bromide to 5.1 g 2-chloro-4-methylpyridine (Loba-Chemie, Vienna) with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloro-4-methylpyridinium bromide. Condition a 1 g C18 SPE cartridge with two 1 mL portions of MeOH and with 1 mL water. Stir 30 mg captopril and 80 mg 1-benzyl-2-chloro-4-methylpyridinium bromide in 5 mL 500 mM pH 8.2 Tris buffer for 1 h, add 1.6 mL 100 mM sodium sulfide, let stand for 10 min, add to the SPE cartridge, wash with two 1 mL portions of water, dry under vacuum for 10 min, wash with three 1 mL portions of MeCN, dry under vacuum for 5 min, elute with 5 mL MeOH: water 80:20. Dilute the eluate to 10 mL with water, dilute with water to an adduct concentration of 3 μg/mL (J. Chromatogr. B 1996, 681, 283). 2-Chloro-4-methylpyridine can also be prepared as follows. Stir 3.6 g 2-amino-4-picoline (2-amino-4-methylpyridine) in 75 g concentrated HCl at between -15° and -20°, saturate with hydrogen chloride gas, add 3.5 g pulverized sodium nitrite in small portions, let stand overnight, neutralize, steam distil to give 2-chloro-4-methylpyridine as a colorless liquid (bp 194-195°) (Ber. 1924, 57, 791).) (27)
Limit of detection: 0.3 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

derivatization; whole blood; SPE

REFERENCE

Sypniewski,S.; Bald,E. Determination of captopril and its disulphides in whole human blood and urine by high-performance liquid chromatography with ultraviolet detection and precolumn derivatization, *J.Chromatogr.A*, **1996**, *729*, 335–340.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 9.652

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve solutions, powders, or powdered tablets in water to give a 200-325 μM solution, filter if necessary. Mix 500 μL solution with 500 μL 170 mM pH 7.5 borate buffer and 500 μL 2 mM 1,1-bis(phenylsulfonyl)ethylene (1,1'-ethenylidenebis(sulfonyl)bis-benzene; Fluka, Merck) in MeOH, let stand at room temperature for 2 min, add 500 μL water, add 300 μL chloroform, vortex for 1 min, centrifuge for 2 min. Remove a 1 mL aliquot of the aqueous layer and add it to 500 μL 300 mM orthophosphoric acid, add 100-200 μL 30 μM IS in MeCN, mix, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:buffer 51:49 (Buffer was 50 mM pH 4.0 triethylamine-phosphate buffer.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3,5
Internal standard: 1(2H)-acenaphthylenone (Synthesis is as follows. Prepare dichloroacenaphthylenone by refluxing acenaphthenequinone with phosphorus pentachloride in anhydrous toluene. Mix 200 g glacial acetic acid, 20 g crude dichloroacenaphthylenone, and 30 g iron powder, heat gently until refluxing, heat for about 3 h, dilute with 500 mL water, steam distil, filter the distillate to recover the product, recrystallize from benzene (Caution! Benzene is a carcinogen!) to obtain 1(2H)-acenaphthylenone (mp 119-121°) (Gazz. Chim. Italia 1938, 68, 184).) (5.5)
Limit of detection: 100 pmole

KEY WORDS
derivatization; tablets; powders

REFERENCE
Cavrini,V.; Gotti,R.; Andrisano,V.; Gatti,R. 1,1'-[Ethylidenebis(sulfonyl)]bis-benzene: A useful pre-chromatographic derivatization reagent for HPLC analyses of thiol drugs, *Chromatographia*, **1996**, *42*, 515–520.

SAMPLE
Matrix: intestinal mucosal homogenate
Sample preparation: 400 μL Homogenate mixture + 400 μL 1 M HCl, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S (Supelco)

Column: 250 × 4.6 Suplecosil LC-18S
Mobile phase: MeOH:water:85% phosphoric acid 54.97:44.98:0.05
Flow rate: 1
Detector: UV 210

KEY WORDS
rat

REFERENCE
Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

SAMPLE
Matrix: solutions
Sample preparation: Add 90 μL cold 10% trichloroacetic acid (containing 1 mM disodium EDTA) to 10 μL blood. Centrifuge at 1850 g at 0° for 5 min. Dilute a 10 μL aliquot of the supernatant with 2 mL water. Mix a 200 μL aliquot of the supernatant with 100 μL 100 μM o-phthalaldehyde reagent and 100 μL 200 μM N-(4-aminobutyl)-N-ethylisoluminol reagent, vortex thoroughly. Let stand for about 2 min. Inject a 20 μL aliquot of the reaction mixture. (Prepare reagents as follows. Dissolve o-phthalaldehyde in 50 mM pH 9.0 sodium borate buffer containing 100 mM potassium dihydrogen phosphate to give a 100 μM solution. Prepare a 1 mM solution of N-(4-aminobutyl)-N-ethylisoluminol (chemiluminescence-grade, Tokyo Kasei) in MeOH containing 5 mM HCl. Dilute with MeOH to give a 200 μM solution.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrosorb RP-18
Column: 150 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque)
Mobile phase: MeOH:THF:100 mM pH 7.5 phosphate buffer 25:5:70
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence, TOA Electronics ICA-3070 detector following post-column reaction. The column effluent mixed with 150 mM hydrogen peroxide pumped at 0.2 mL/min and 25 μM hematin in 150 mM sodium carbonate buffer pumped at 3 mL/min and this mixture flowed through a 200 × 0.5 mm PTFE reaction coil to the detector.

CHROMATOGRAM
Retention time: k' 13.3

OTHER SUBSTANCES
Extracted: acetylcysteine

KEY WORDS
post-column reaction; derivatization

REFERENCE
Sano,A.; Nakamura,H. Chemiluminescence detection of thiols by high-performance liquid chromatography using o-phthalaldehyde and N-(4-aminobutyl)-N-ethylisoluminol as precolumn derivatization reagents, *Anal.Sci.*, **1998**, *14*, 731–735.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 200 μM solution in buffer with three volumes of a 400 μM solution of 5,5'-dithio-(bis-2-nitrobenzoic acid) in buffer, let stand at room temperature for 30 min, inject a 75 μL aliquot. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS1
Mobile phase: Gradient. MeCN:buffer 0:100 for 20 min, to 17.5:82.5 over 40 min. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)
Flow rate: 0.25 for 20 min, to 1 over 40 min
Injection volume: 75
Detector: UV 357

CHROMATOGRAM
Retention time: 61

OTHER SUBSTANCES
Simultaneous: N-acetylcysteine, N-acetylpenicillamine, cysteine, glutathione, penicillamine, thiomalic acid

KEY WORDS
derivatization

REFERENCE
Russell,J.; McKeown,J.A.; Hensman,C.; Smith,W.E.; Reglinski,J. HPLC determination of biologically active thiols using pre-column derivatization with 5,5'-dithio-(bis-2-nitrobenzoic acid), *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1757–1763.

SAMPLE
Matrix: urine
Sample preparation: Dilute 10 mL urine to 15 mL with water, add to Extrelut-20 SPE cartridge, elute with 60 mL ethyl acetate:isopropanol 85:15. Evaporate under vacuum at 50°, filter, dry under nitrogen, reconstitute the residue in 100 μL acetone. Add 100 μL 1 mg/mL 3-bromomethylpropylphenazone in acetone, mix with 1 mg anhydrous potassium carbonate, make up to 200 μL with acetone. Let stand at 105 ± 5° for 30 min (for captopril) or 60 min (for hydrochlorothiazide or for the mixture). Cool the reaction mixture, dry under a gentle stream of nitrogen. Reconstitute the residue with 500 μL MeCN, shake for 2 min. inject a 10 μL aliquot. (3-Bromomethylpropylphenazone is produced by the reaction of propylphenazone with bromine and recrystallized from chloroform:diethyl ether 1:2. (Caution! Chloroform is a carcinogen!))

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeCN:MeOH:50 mM sodium acetate 34:8:28, adjusted to pH 6.5 with acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 254

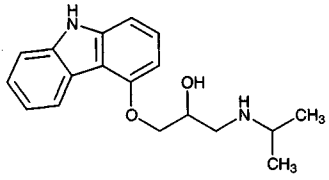
CHROMATOGRAM
Retention time: 8.6

OTHER SUBSTANCES
Extracted: hydrochlorothiazide

KEY WORDS
derivatization; SPE

REFERENCE
Khedr,A.; El-Sherief,H. 3-Bromomethyl-propyphenazone as a new derivatization reagent for high performance liquid chromatography of captopril and hydrochlorothiazide with UV-detection, *Biomed.Chromatogr.*, **1998**, *12*, 57–60.

Carazolol



Molecular formula: $C_{18}H_{22}N_2O_2$
Molecular weight: 298.38
CAS Registry No.: 57775-29-8
Merck Index: 1822

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 µL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 µL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES

Guard column: 10 × 2.1 37-50 µm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: F ex 246 em 351

CHROMATOGRAM

Retention time: 5
Limit of detection: 0.3 ng/g

OTHER SUBSTANCES

Extracted: azaperol, chlorpromazine, acepromazine, xylazine, azaperone, haloperidol, propiomazine

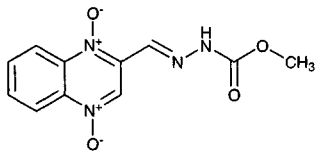
KEY WORDS

SPE; pig; kidney

REFERENCE

Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

Carbadox



Molecular formula: $C_{11}H_{10}N_4O_4$
Molecular weight: 262.22
CAS Registry No.: 6804-07-5
Merck Index: 1825

SAMPLE

Matrix: blood, eggs, tissue

Sample preparation: Blend 10 g muscle, liver, kidney, plasma, or eggs with 40 mL MeCN:MeOH for 3 min (stomacher blender), centrifuge at 2000 g for 5 min, pass the supernatant through a 400 × 10 glass column containing 8 g alumina (Woelm neutral, activity 1, lower layer) and 2 g florisil (75-150 μm, upper layer), collect the eluate. Evaporate 10 mL of the eluate to 0.9-1.1 mL under a stream of nitrogen at 40-50°, dilute to 4 mL with water, mix, extract with 2 mL isooctane, centrifuge at 2000 g for 5 min, inject 1 mL of the aqueous phase onto column A with mobile phase A, after 20 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 2.1 column was slurry-packed with 55-105 μm material from a C18 Sep-Pak, 20 μm screens were used; B 10 × 2.1 37-50 μm Bondapak C18/Corasil + 100 × 3 5 μm ChromSpher C18 (Chrompack)

Mobile phase: A water; B MeCN:10 mM pH 6 acetate buffer 14:86

Flow rate: A 0.3; B 0.5

Injection volume: 1000

Detector: UV 390 following post-column derivatization. The column effluent was mixed with 0.5 M NaOH pumped at 0.23 mL/min. The mixture flowed through a 2 m × 0.5 mm i.d. knitted PTFE reaction coil to the detector.

CHROMATOGRAM

Retention time: 9

Limit of detection: 1 ng/g

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: chloramphenicol, chlortetracycline, clopidol, dapsone, decoquinate, dimetridazole, dinitolmide, doxycycline, ethopabate, fenbendazole, furaltadone, furazolidone, furnicozone, halofuginone, ipronidazole, methylbenzoquate, nicarbazin, nifursol, nitrofurantoin, nitrofurazone, nitrovin, olaquindox, oxytetracycline, pyrantel, robenidine, ronidazole, sulfadiazine, sulfanilamide, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, tetracycline, thiophanate, trimethoprim

KEY WORDS

plasma; column-switching; post-column reaction; derivatization; use yellow light and amber glassware; muscle; liver; kidney; pig

REFERENCE

Binnendijk,G.M.; Aerts,M.M.L.; Keukens,H.J.; Brinkman,U.A.T. Optimization and ruggedness testing of the determination of residues of carbadox and metabolites in products of animal origin. Stability studies in animal tissues, *J.Chromatogr.*, **1991**, *541*, 401–410.

SAMPLE

Matrix: feed

Sample preparation: Grind feed to pass 20 mesh. 10 g Feed + 5 mL water, swirl, let stand for 5 min, add 50 mL DMF:water 95:5, shake vigorously for 15 s, let stand in the dark

at room temperature overnight, filter (paper). Add 15 mL of the filtrate to 5 g alumina (Alcoa F-20, 80-200 mesh) in a 300 × 10 glass column, discard first several mL of eluate, collect remaining eluate, inject an aliquot

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:1% acetic acid 20:80
Detector: UV 280, UV 365

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: furazolidone, nitrofurazone

KEY WORDS
protect from light

REFERENCE
Thorpe,V.A. Sample preparation of carbadox, furazolidone, nitrofurazone, and ethopabate in medicated feeds for high pressure liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 981–984.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh sieve. 10 g Ground feed + 15 mL water, mix well, let stand for 5 min, add 25 mL MeCN:MeOH 50:50, shake vigorously for 30 min, centrifuge or filter (Whatman glass fiber GFA), pass 15 mL supernatant or filtrate through 4 g alumina in a 10 mm dia column, collect the first 4 mL eluate, inject a 25 μL aliquot. (Prepare alumina as follows. Stir 200 g Fisher neutral alumina (A-950) in 1 L water for 30 min, pour off fines, resuspend, filter (Whatman glass fiber GFA), dry with vacuum, wash 3 times with MeOH, dry at 80° overnight, store in desiccator.)

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Waters)
Column: 100 × 5 C18 radial compression (Waters)
Mobile phase: MeCN:buffer 18:82 (Buffer was 25 mL dibutylamine acetate made up to 1 L with water, pH 3.7. Dibutylamine acetate was prepared by titrating 100 mL dibutylamine to pH 2.5 with acetic acid (ca. 270 mL).)
Flow rate: 2
Injection volume: 25
Detector: UV 365

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: pyrantel (UV 313)

KEY WORDS
protect from light

REFERENCE
Lowie,D.M.,Jr.; Teague,R.T.,Jr.; Quick,F.E.; Foster,C.L. High pressure liquid chromatographic determination of carbadox and pyrantel tartrate in swine feed and supplements, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 602–605.

SAMPLE

Matrix: feed

Sample preparation: Grind feed to pass 2 mm sieve. 50 g Ground feed + 200 mL extraction solution, shake mechanically for 30 min, centrifuge at 1500 rpm for 5 min. Add 100 mL of the supernatant to a Celite column, rinse apparatus with 70 mL extraction solution, add rinses to Celite column. Collect all the eluates and add them to alumina column A, rinse apparatus with 20-30 mL extraction solution, add rinses to alumina column A. Collect all the eluate and evaporate just to dryness under reduced pressure, take up the residue in 50 mL chloroform. Extract with 10 mL 1 M NaOH (30 s shake), add 12 mL 1 M HCl to the aqueous phase, extract three times with 50 mL chloroform. Combine the extracts and dry them over 35-40 g anhydrous sodium sulfate. Evaporate to dryness and take up the residue in 5 mL DMF, add to alumina column B, rinse apparatus with 10 mL DMF, add rinses to column, wash with three 10 mL portions of DMF, wash with three 25 mL portions of chloroform, elute with chloroform:MeOH 75:25. Evaporate the eluate just to dryness, take up the residue in 2 mL MeOH:water 30:70, inject a 20 μL aliquot. (Extraction solution was chloroform:acidified MeOH 75:25. Acidified MeOH was MeOH containing 1% HCl. The Celite column was a 300 × 22 glass column containing 8-10 g acid-washed Celite 545, prewet with 50 mL extraction solution. Alumina column A was a 400 × 22 glass column containing 15 cm of alumina (Fisher A-540, 80-200 mesh), prewet with 50 mL extraction solution. Alumina column B was a 300 × 22 glass column containing 3 cm of alumina (Fisher A-540, 80-200 mesh), prewet with 25 mL DMF.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve spherical C18 (Waters)
Mobile phase: MeOH:water 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM

Retention time: 4.7
Limit of quantitation: 10 ppb

OTHER SUBSTANCES

Simultaneous: furazolidone, nitrofurazone
Noninterfering: pyrantel

KEY WORDS

protect from light; SPE

REFERENCE

Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of carbadox residues in animal feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 653–657.

SAMPLE

Matrix: feed

Sample preparation: Blend 10-100 g feed with 200 mL chloroform:MeOH 75:25 for 3 min, filter through 25 mm Celite 545, re-extract residue with 200 mL chloroform:MeOH 75:25, add 50 mL chloroform:MeOH 75:25 to the residue, filter this mixture, wash the filter cake with several small portions of chloroform:MeOH 75:25. Evaporate the filtrate on a steam bath with a current of air to about 100 mL. Shake the organic layer with 50 mL 10% NaCl in 100 mM NaOH for 1 min, wash the aqueous phase with two or three 50 mL portions of chloroform (until wash is colorless), add 10 mL 1 M KH_2PO_4 solution to the aqueous phase, extract with three 50 mL portions of chloroform. Combine the extracts and filter them through a 25 mm layer of sodium sulfate, evaporate most of the filtrate on a steam bath with a current of air, evaporate the remainder with a current of air. Take

up the residue in 100 mL MeOH, remove a 20 mL aliquot, evaporate most on a steam bath with a current of air, evaporate the remainder with a current of air, dissolve the residue in 100 μL MeOH, make up to 10 mL with MeCN:water 25:75, filter (0.45μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.5% acetic acid and 0.05% sodium 1-octanesulfonate.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: sulfamethazine

REFERENCE
McGary,E.D. Quantitative determination of sulphamethazine and carbadox in animal feeds by paired ion high-performance liquid, *Analyst*, **1986**, *111*, 1341–1342.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20-mesh sieve. Add 10 g feed to 10 mL water with swirling, let stand for 5 min (make sure all particles are wet), add 50 mL DMF: water 95:5, shake vigorously for 15 s, shake on a wrist action shaker for 30 min, filter (paper), pass 15 mL through 8 g alumina in a 300 × 10 glass column, if necessary dilute eluate with DMF:water 95:5, inject a 20 μL aliquot. (The alumina was Alcoa F-20, 80-200 mesh, Sargeant-Welch No. SC 10492-005LB, do not substitute.)

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: Partisil 10 ODS-3
Mobile phase: DMF:buffer 23.5:76.5 (22.3 g $Na_4P_2O_7.H_2O$ and 50 mL phosphoric acid in 700 mL water, add 235 mL DMF, adjust pH to 1.9 ± 0.1 with phosphoric acid , make up to 1 L. Shake DMF with activated carbon and filter before use.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: morantel, pyrantel
Noninterfering: lincomycin, tylosin, furazolidone, nitrofurazone, ethopabate, amprolium, sulfa drugs, chlortetracycline, oxytetracycline

REFERENCE
Thorpe,V.A. A collaborative study: high-pressure liquid chromatographic determination of carbadox and pyrantel tartrate in animal feeds, *J.Chromatogr.Sci.*, **1988**, *26*, 545–550.

SAMPLE
Matrix: feed

Sample preparation: Grind and sieve feed with a Moulinex blender. Weigh out 10 g and add it to 20 mL DMF and 60 mL carbon tetrachloride, stir magnetically at 500 rpm at 60° for 30 min, cool, filter (100 μm glass), wash the residue with a little carbon tetrachloride. Remove 25 mL of the filtrate and add it to 45 mL water, stir vigorously for 2 min, centrifuge at 320 g for 5 min, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: Gradient. MeOH:water 15:85 for 4 min, to 50:50 over 2 min, maintain at 50:50 for 4 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 305

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Extracted: olaquindox (UV 262 nm)

KEY WORDS

protect from light

REFERENCE

dos Ramos,F.J.; da Silveira,I.N.; de Graaf,G. Column liquid chromatographic determination of carbadox and olaquindox in feeds, *J.Chromatogr.*, **1991**, *558*, 125–130.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in chloroform at a concentration of 1 μg/mL, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrospher RP-18
Mobile phase: MeCN:10 mM sodium acetate 20:80, pH 5
Column temperature: 30
Flow rate: 1.6
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: 5.9
Limit of detection: 11 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, nitrofurazone, nitrofurantoin, furazolidone, furaltadone

REFERENCE

Kaniou,I.; Zachariadis,G.; Kalligas,G.; Tsoukali,H.; Stratis,J. Separation and determination of carbadox, nitrofurazone, nitrofurantoin, furazolidone, and furaltadone in their mixtures by thin layer and high performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 1385–1398.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Waring blender) 10 g muscle, liver, or kidney in 100 mL EtOH for 5 min, let stand for 5 min, filter through 10 g Celite 545 on top of a sintered

glass filter, rinse blender with 100 mL EtOH and filter rinse. Add 25 mL 3.6% aqueous metaphosphoric acid to the combined filtrates, evaporate to 25 mL under reduced pressure at 45°. Remove residue, rinse out flask with 5 mL hexane and 3 mL water, combine, centrifuge at 0° at 27000 g for 30 min, discard hexane, rinse surface with 5 mL hexane, discard hexane. Remove aqueous layer, rinse out tube twice with 3 mL portions of water, combine, add 10 mL 1 M KH_2PO_4, make up to 100 mL with water, extract three times for 5 min with 50 mL ethyl acetate. Combine the extracts and dry them over 15 g anhydrous sodium sulfate, filter through glass wool, evaporate to dryness under reduced pressure at 45°. Take up residue in 3 mL ethyl acetate and add to alumina column, rinse flask with 2 mL ethyl acetate and add rinse to column. Elute with 20 mL EtOH:MeOH: ethyl acetate 10:10:80 and combine all the eluate. Evaporate to dryness under reduced pressure at 45°, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot. (Prepare alumina column by slurrying 1 g aluminum oxide (Baker) in 20 mL ethyl acetate and adding to a 200 × 6 glass chromatographic column.)

HPLC VARIABLES

Guard column: Brownlee 10 μm RP-GU MPLC C-8
Column: 250 × 4.6 Brownlee RP-10A C-8
Mobile phase: MeCN:EtOH:10 mM ammonium acetate 25:5:70, pH 6.8
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 5.0
Limit of detection: 2 ng
Limit of quantitation: 10 ng

OTHER SUBSTANCES

Extracted: quinoxaline-2-carboxylic acid, furazolidone, nitrofurazone, desoxycarbadox

KEY WORDS

protect from light; pig; muscle; liver; kidney

REFERENCE

MacIntosh,A.I.; Neville,G.A. Liquid chromatographic determination of carbadox, desoxycarbadox, and nitrofurazones in pork tissues, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 958–962.

SAMPLE

Matrix: tissue
Sample preparation: Blend (stomacher) 10 g tissue with 40 mL MeCN:MeOH for 3 min, centrifuge at 2000 g for 5 min, add the supernatant to a 400 × 10 column having 2 g 75-150 μm Florisil on top of 8 g alumina (Woelm neutral, activity 1). Collect the first 10 mL of eluate and evaporate it to 1-1.5 mL under a stream of nitrogen at 40-50°, make up to 4 mL with water, mix, add 2 mL isooctane, extract, centrifuge at 2000 g for 5 min, inject a 2 mL aliquot of the aqueous phase onto column A and elute to waste with mobile phase A, after 20 min backflush the contents of column A onto column B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4.6 37-50 μm Bondapak C18/Corasil; B 10 × 2.1 37-50 μm Bondapak C18/Corasil + 200 × 3 5 μm ChromSpher C18 (Chrompack)
Mobile phase: A Water; B MeCN:10 mM pH 6 sodium acetate buffer 15:85
Flow rate: A 0.5; B 0.6
Injection volume: 2000

Detector: UV 420 following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.23 mL/min and the mixture flowed through a 2 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: 6
Limit of detection: 0.5-1 ng/g

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: furaltadone, furazolidone, nitrofurantoin, nitrofurazone
Noninterfering: chloramphenicol, chlortetracycline, clopidol, dapsone, decoquinate, dimetridazole, dinitolmide, doxycycline, ethopabate, fenbendazole, furnicozone, halofuginone, ipronidazole, methylbenzoquate, nicarbazin, nifursol, nitrovin, olaquindox, oxytetracycline, pyrantel, robenidine, ronidazole, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, tetracycline, thiophanate, trimethoprim

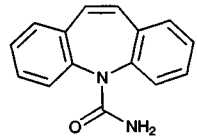
KEY WORDS

post-column reaction; column-switching; protect from light; muscle; liver; kidney; pig; SPE

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Keukens,H.J.; Brinkman,U.A.T. Determination of residues of carbadox and some of its metabolites in swine tissues by high-performance liquid chromatography using on-line pre-column enrichment and post-column derivatization with UV-VIS detection, *J.Chromatogr.*, **1988**, *456*, 105–119.

Carbamazepine



Molecular formula: $C_{15}H_{12}N_2O$
Molecular weight: 236.27
CAS Registry No.: 298-46-4
Merck Index: 1826
Lednicer No.: 1 403

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 11.5
Internal standard: cyheptamide (14)
Limit of detection: 15-30 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine diol, carbamazepine epoxide, lamotrigine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, phenytoin
Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum; SPE

REFERENCE

Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE

Matrix: blood
Sample preparation: Mix 500 μL plasma with 850 μL MeCN, vortex, centrifuge at 1500 g for 5 min. Inject an aliquot.

HPLC VARIABLES
Column: 33 × 4.6 1.5 μm Kovasil MS C14 (CU Chemie Uetikon, Switzerland)
Mobile phase: MeCN:20 mM pH 7.0 potassium phosphate buffer 7:93
Column temperature: 60
Flow rate: 1.2
Injection volume: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 2.27
Limit of quantitation: 4 μM

OTHER SUBSTANCES
Simultaneous: metabolites, clobazam, desmethylclobazam, ethosuximide, felbamate, pheneturide, (±)-5-(p-hydroxyphenyl)-5-phenylhydantoin, 5-(m-hydroxyphenyl)-5-phenylhydantoin phenobarbital, phenylethylmalonamide, phenytoin, primidone
Noninterfering: vigabatrin, valproic acid

KEY WORDS
plasma

REFERENCE
Chollet,D.; Castella,E.; Combe,P.; Arnera,V. High-performance liquid chromatographic method for the monitoring of carbamazepine and its active metabolite, carbamazepine-10,11-epoxide, in human plasma, *J.Chromatogr.B*, **1996**, *683*, 237–243.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood

Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 9.0
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, phenobarbital, phenytoin, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: Dilute 20 μL serum with 100 μL pH 3.7 phosphate buffer, shake vigorously for 10 s, add to a 45 μL PTFE column packed with 50 μm ODS-silica (Asahi Chemicals, Tokyo) (Extrashot-ODS device), wash with 100 μL water, elute with 130 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 4.4 potassium phosphate buffer 14:21:65
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 18.7

OTHER SUBSTANCES
Extracted: phenobarbital, phenytoin

KEY WORDS
SPE

REFERENCE
Kouno,Y.; Ishikura,C.; Homma,M.; Oka,K. Extrashot-ODS, a syringe-type minicolumn sample injector for a reversed-phase high-performance liquid chromatographic column. Application to antiepileptics in human sera, *J.Chromatogr.B*, **1997**, *695*, 349–353.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 40 μg/mL 5-ethyl-5-p-tolylbarbituric acid and 3 mL dichloromethane to 100 μL plasma, vortex for 2 min, centrifuge at 1200 g for 5 min, evap-

orate the organic phase under a gentle nitrogen stream in a water bath at 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS C18
Mobile phase: MeCN:water 30:70
Flow rate: 0.4
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 9.7
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (8.8)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat

REFERENCE
Tanaka,E. Simultaneous determination of carbamazepine and its metabolites in plasma from carbon tetrachloride-intoxicated rats using a new reversed-phase chromatographic column of 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *688*, 155–160.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5-20 μl aliquot onto the column with mobile phase A or B. Urine. Inject a 20 μL aliquot onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 20:80 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:86 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum); UV 230 (urine)

CHROMATOGRAM
Retention time: 7.61 (serum, A), 15.5 (serum, B), 17.5 (urine,C)
Limit of detection: 3 ng (urine)

OTHER SUBSTANCES
Simultaneous: acetaminophen (B), barbital (B), phenobarbital (B,C), phenytoin (B,C), primidone (B,C), sulfapyridine (A,B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 213.4

CHROMATOGRAM

Retention time: 15.763

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Zorbax SB C18

Mobile phase: Gradient. MeCN:20 mM pH 7.5 phosphate buffer from 30:70 to 40:60 over 10 min. (Mobile phase was contained 0.1% triethylamine.)

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: omeprazole

REFERENCE

Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco LC-8
Mobile phase: MeOH:100 mM K_3PO_4 45:55 containing 100 μL/L triethylamine

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Cohen,H.; Howland,M.A.; Luciano,D.J.; Rubin,R.N.; Kutt,H.; Hoffman,R.S.; Leung,L.K.H.; Devinsky,O.; Goldfrank,L.R. Feasibility and pharmacokinetics of carbamazepine oral loading doses, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1134–1140.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 285

OTHER SUBSTANCES
Also analyzed: fenbufen, indomethacin, ketoprofen, α.-naphthoquinone, naproxen, tolmetin

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.74

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

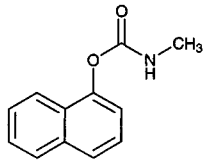
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Carbaryl



Molecular formula: $C_{12}H_{11}NO_2$
Molecular weight: 201.22
CAS Registry No.: 63-25-2
Merck Index: 1831

SAMPLE

Matrix: beverages
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 4 mL MeOH and 5 mL water. Pass 60 mL juice through the cartridge, wash with 5 mL MeCN:water 25:75, elute with 2 mL MeCN:water 75:25, inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultremex
Mobile phase: MeCN:MeOH:water 15:40:45
Flow rate: 1
Injection volume: 20-50
Detector: UV 224

CHROMATOGRAM

Retention time: 4
Limit of detection: 5 ppb

KEY WORDS

fruit juice; SPE

REFERENCE

Bushway,R.J. High-performance liquid chromatographic determination of carbaryl in fruit juices, *J.Chromatogr.*, **1988**, *457*, 437–441.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 12.89
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize tissue with an equal volume of water, treat with a saturated solution of calcium chloride, let stand overnight, filter. Extract filtrate, blood, or other body fluid with an equal volume of ether. Adjust pH of aqueous layer to 2 with 2 M HCl, extract with an equal volume of ether. Combine the ether layers, evaporate to dryness, reconstitute in a suitable solvent, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax cyano
Mobile phase: Iso-octane:ethyl acetate 80:20
Flow rate: 1
Injection volume: 20
Detector: RI

CHROMATOGRAM

Retention time: 8.70
Limit of detection: 100 ng

OTHER SUBSTANCES

Extracted: methyl parathion, dichlorvos, monocrotophos, quinalphos, malathion, phosphamidon, propoxur (baygon)

KEY WORDS

liver; lung

REFERENCE

Sharma,V.K.; Jadhav,R.K.; Rao,G.J.; Saraf,A.K.; Chandra,H. High performance liquid chromatographic method for the analysis of organophosphorus and carbamate pesticides, *Forensic Sci.Int.*, **1990**, *48*, 21–25.

SAMPLE

Matrix: blood, urine

Sample preparation: Evaporate 25 μL of a 6.5 μg/mL solution of napropamide into a tube, add 250 μL whole blood, plasma, or urine, add 250 μL water, wait for 5-10 min, add 2.5 mL ethyl acetate, shake for 10 min, centrifuge at 1200 g for 8 min. Remove 2 mL of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL EtOH:MeCN:130 mM pH 6.2 phosphate buffer 50:20:30, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8 (Alltech)
Mobile phase: MeCN:130 mM pH 6.2 phosphate buffer 40:60
Flow rate: 1.42
Injection volume: 50-100
Detector: F ex 285 em 340 (cut-off filter)

CHROMATOGRAM
Retention time: 10.6
Internal standard: napropamide (30)
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 1-naphthol

KEY WORDS
whole blood; plasma

REFERENCE
DeBerardinis,M.,Jr.; Wargin,W.A. High-performance liquid chromatographic determination of carbaryl and 1-naphthol in biological fluids, *J.Chromatogr.*, **1982**, *246*, 89–94.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 17.968

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: food

Sample preparation: 30 g Rice + 50 mL MeOH, let stand for 48 h with occasional manual shaking, inject a 10 μL aliquot of the MeOH layer. Alternatively, remove a 1 mL aliquot of the MeOH layer and evaporate it to near dryness under a stream of nitrogen, add 1 mL hexane, shake, repeat extraction. Combine the hexane layers and add them to a Sep-Pak Florisil SPE cartridge, elute with 3 mL acetone:hexane 40:60. Combine the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH, inject a 10 μL aliquot. (Recovery is 97% with direct injection or 30% with sample clean-up.)

HPLC VARIABLES

Guard column: Guard-Pak (Waters)

Column: 250 × 3.9 Nova-Pak C18

Mobile phase: Gradient. MeCN:water from 40:60 to 70:30 over 12 min or Isocratic (1) MeCN:water 60:40 or Isocratic (2) MeCN:water 40:60

Flow rate: 1

Injection volume: 10

Detector: UV 225

CHROMATOGRAM

Retention time: 4 (gradient), 2 (isocratic 1), 7 (isocratic 2)

Limit of detection: 50 ng/g (without clean-up)

OTHER SUBSTANCES

Extracted: methacrifos, fenitrothion, etrimfos, chlorpyrifos-methyl, pirimphos-methyl (UV 247) (with gradient or isocratic 1)

KEY WORDS

rice; SPE

REFERENCE

Brayan,J.G.; Haddad,P.R.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *447*, 249–255.

SAMPLE

Matrix: food

Sample preparation: Prepare a column by adding 500 mg silanized Celite 545 to a 22 mm ID column, add 5 g Nuchar S-N:silanized Celite 545 20:80, wash with 50 mL MeCN:toluene 75:25, do not allow to go dry. Homogenize (Polytron) 150 g sample with 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter, remove a portion equivalent to 100 g sample, add water so that there is 100 mL water in the flask, concentrate to 75 mL under reduced pressure at 35°, add 15 g NaCl, add 75 mL MeCN, shake for 30 s, extract the aqueous layer with 50 mL MeCN for 20 s. Combine the organic layers and add them to 25 mL 20% NaCl, shake, discard the aqueous layer, wash the MeCN layer with 100 mL petroleum ether, extract the petroleum ether layer with 10 mL MeCN. Combine the MeCN layers and add 50 mL 2% NaCl, extract with 100 mL dichloromethane, extract twice with 25 mL portions of dichloromethane, filter the dichloromethane layers through 5 g anhydrous sodium sulfate, evaporate the dichloromethane extracts to dryness under reduced pressure, immediately reconstitute with 10 mL dichloromethane, add to the column, elute at 5 mL/min, rinse flask with 10 mL dichloromethane, add the rinse to the column, elute with 125 mL MeCN:toluene 75:25, collect all the effluent from the column and evaporate it just to dryness under reduced pressure, reconstitute with 5 mL MeOH, filter (5 μm), inject an aliquot. (Silanize Celite 545 by boiling 150 g Celite 545 in 1 L HCl:water 50:50 for 10 min, cool, filter, wash with water until the filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry, heat to 120°, cool

in desiccator, add 3 mL dichlorodimethylsilane, mix well, let stand at room temperature for 4 h, add 500 mL MeOH, mix, let stand for 15 min, filter, wash with isopropanol until neutral, air dry, dry at 105° for 2 h, cool in desiccator. Test for total silanization by placing 1 g in 20 mL toluene saturated with methyl red. Silanized Celite should appear yellow. Silanized Celite should also float on water. If silanization is not complete, repeat process. Boil 100 g Nuchar S-N with 700 mL HCl for 1 h, add 700 mL water, boil for 30 min, cool, filter, wash with water until the filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry, dry at 120° for 4 h, cool in desiccator.) (J. Assoc. Off. Anal. Chem. 1985, 68, 726).

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Perisorb RP-8
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 20:80 to 70:30 over 25 min, re-equilibrate at initial conditions for 10 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: E, ESA Model 5100A, Model 5010 dual analytical cell, detector 1 + 0.20 V, detector 2 +0.60 V (monitored), Model 5020 guard cell in NaOH stream at +0.70 V, following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.5 ± 0.02 mL/min and the mixture flowed through a 3 m × 0.48 mm ID stainless-steel coil at 100° to the detector.

CHROMATOGRAM
Retention time: 15
Limit of detection: 0.25 ng
Limit of quantitation: 10 ppb

OTHER SUBSTANCES
Extracted: bufencarb, carbofuran, 3-hydroxycarbofuran, isoprocarb, methiocarb

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KEY WORDS
post-column reaction; apples; cabbages; grapes; tomatoes

REFERENCE
Krause,R.T. High-performance liquid chromatographic determination of aryl N-methylcarbamate residues using post-column hydrolysis electrochemical detection, *J.Chromatogr.*, **1988**, *442*, 333–343.

SAMPLE
Matrix: fruit
Sample preparation: Blend 50 g chopped grapes with 100 mL MeCN, 25 mL water, and 10 g Celite for 15 min, filter (fritted glass). Dilute the filtrate with MeCN:water 50:50, evaporate a 10 mL aliquot to dryness under reduced pressure, reconstitute with 3 mL MeOH, filter (0.2 μm Nylon), wash the filter with 2 mL MeOH, evaporate the filtrate to 1 mL, dilute with 5 mL THF, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5 amino
Mobile phase: Gradient. THF:10 mM tetramethylammonium hydrogen sulfate:50 mM pH 6.7 acetate buffer 99.8:0.2:0 for 10 min, to 98.9:0.1:1 over 1 min, maintain at 98.9:0.1: for 9 min
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 333

CHROMATOGRAM
Retention time: 3.80

Limit of detection: 20 ng/g
Limit of quantitation: 70 ng/g

OTHER SUBSTANCES

Extracted: aminocarb (F ex 255 em 367), carbendazim (F ex 285 em 318), fuberidazol (F ex 310 em 345), 1-naphthylacetamide (F ex 282 em 340)

KEY WORDS

grapes

REFERENCE

García Sńchez,F.; Navas Díaz,A.; García Pareja,A. Normal phase liquid chromatography on amino-bonded-phase column of fluorescence detected pesticides, *J.Liq.Chromatogr.*, **1995**, *18*, 4543–2558.

SAMPLE

Matrix: fruit, grain, vegetables

Sample preparation: Homogenize (Polytron) 150 g high moisture sample and 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter (paper) under vacuum, remove portion of filtrate equal to 100 g sample and make up to 100 mL with water. Homogenize (Polytron) 75 g low moisture sample and 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter (paper) under vacuum, remove portion of filtrate equal to 50 g sample and make up to 100 mL with water. Concentrate samples to 75 mL under reduced pressure at 35°, add 15 g NaCl, add 75 mL MeCN, shake for 30 s, let stand for 5 min. Remove the aqueous phase and add it to 50 mL MeCN, shake for 20 s, let layers separate, discard the aqueous layer. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, extract petroleum ether layer with 10 mL MeCN. Combine the MeCN layers and add them to 50 mL 2% NaCl, extract with 100 mL dichloromethane, extract twice with 25 mL portions of dichloromethane. Combine the dichloromethane layers and pass them through a 22 mm i.d. column containing 5 g anhydrous sodium sulfate. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute in 10 mL dichloromethane, add to the charcoal column, rinse flask with 10 mL dichloromethane, rinse flask with 25 mL MeCN:toluene 75:25. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute with 5 mL MeOH, filter (5 μm), inject a 10 μL aliquot (J. Assoc. Off.Anal. Chem. 1980, 63, 1114). (Charcoal column was 5 g silanized Celite 545: Nuchar S-N 4:1 on top of 0.5 g silanized Celite 545 in a 300 × 22 glass column, wash with 50 mL MeCN:toluene 75:25, do not allow to go dry. Prepare silanized Celite 545 as follows. Boil 150 g Celite 545 in 1 L 6 M HCl with stirring for 10 min, cool, filter, wash with water until filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry in hood, heat to 120° in a flask, cool in a desiccator, add 3 mL dichlorodimethylsilane, mix well, let stand at room temperature for 4 h, add 500 mL MeOH, mix, let stand for 15 min, filter, wash with isopropanol until neutral, air dry in hood, dry at 105° for 2 h, cool in desiccator, store in stoppered container. Totally silanized Celite should float on water and appear yellow (not pink) in toluene saturated with methyl red (J. Assoc. Off.Anal. Chem. 1980, 63, 1114).)

HPLC VARIABLES

Guard column: 70 × 2.1 25-37 μm Co-Pell ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 12:88 to 70:30 over 30 min, 100:0 for 5 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: F ex 288 em 330 following post-column reaction. The column effluent mixed with 200 mM NaOH pumped at 0.5 mL/min and flowed through a 3 m × 0.48 mm stainless steel column to the detector.

CHROMATOGRAM

Retention time: 21

Limit of quantitation: 20 ppb

OTHER SUBSTANCES
Extracted: carbofuran, napropamide, phosalone, piperonyl butoxide

KEY WORDS
post-column reaction; pears; green beans

REFERENCE
Krause,R.T.; August,E.M. Applicability of a carbamate insecticide multiresidue method for determining additional types of pesticides in fruits and vegetables, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 234–240.

SAMPLE
Matrix: fruit, vegetables

Sample preparation: Homogenize (Omni-Mixer) 100 g chopped sample with 250 mL MeOH at half-speed for 5 min, filter (Whatman No. 1 PS paper), make up filtrate to 500 mL with MeOH. Remove 100 mL filtrate and add it to 125 mL 4% aqueous sodium sulfate, shake well, extract mixture with 75, 50, and 50 mL portions of dichloromethane with 30 s shaking each time, drain organic layers through anhydrous sodium sulfate. Combine the organic layers and evaporate them to 1 mL under reduced pressure at 30°, transfer residue to a tube with two 2 mL rinses of dichloromethane:cyclohexane 50:50, make volume up to 10 mL with dichloromethane:cyclohexane 50:50, filter (0.45 μm), add 5 mL to a 600 × 25 tube containing 60 g 200-400 mesh BioBeads SX-3 resin (Analytical Bio-Chemistry Laboratories), pump through at 5 mL/min with dichloromethane:cyclohexane 50:50 mobile phase, discard mobile phase for 24 min, collect fraction containing the compound for 12 min, evaporate under reduced pressure at 30° to low volume, add 15 mL MeOH, evaporate to about 1 mL, filter (0.45 μm), inject a 20 μL aliquot. Alternatively, run output from BioBeads column through a column containing 0.5 g of a mixture of Nuchar S-N(Fisher):Celite 545 1:4, at the end of the chromatography elute this column with 10 mL MeCN:toluene 75:25, evaporate the eluate under reduced pressure at 30° to low volume, add 15 mL MeOH, evaporate to about 1 mL, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Pellicular ODS (Whatman)

Column: 250 × 4.6 5 μm Apex ODS (Jones Chromatography)

Mobile phase: Gradient. MeOH:water from 10:90 to 90:10 over 23 min, to 10:90 over 4 min, re-equilibrate at 10:90 for 10 min

Flow rate: 1

Injection volume: 20

Detector: F ex 340 em 455, following post-column derivatization. The column effluent is mixed with 200 mM NaOH at 0.8 mL/min and the mixture flows through a 1 mL coil at 95° and is mixed with 500 mg/L o-phthalaldehyde and 1 mL/L 2-mercaptoethanol in 50 mM sodium tetraborate pumped at 0.8 mL/min. The mixture flows through a 0.5 mL coil at ambient temperature to the detector.

CHROMATOGRAM
Retention time: 25

Limit of detection: 5-10 ppb

OTHER SUBSTANCES
Extracted: oxamyl, methomyl, aldicarb, propoxur, carbofuran, methiocarb

KEY WORDS
apples; broccoli; cabbage; cauliflower; potatoes; post-column reaction; derivatization

REFERENCE

Chaput,D. Simplified multiresidue method for liquid chromatographic determination of N-methyl carbamate insecticides in fruits and vegetables, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 542–546.

SAMPLE

Matrix: soil

Sample preparation: 100 g Soil + 100 mL water, blend, add 125 mL MeOH, blend for 5 min, centrifuge for 15 min, let stand for 10 min. Remove the supernatant and extract the residue with 50 mL MeOH. Combine the supernatants and extract them three times with 100 mL portions of dichloromethane, dry the organic layers over anhydrous sodium sulfate. Evaporate the dichloromethane under vacuum at 32-35°, take up the residue in mobile phase, clean up further using a Sep-Pak SPE cartridge, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 60:40
Flow rate: 1
Injection volume: 5
Detector: UV 224

CHROMATOGRAM

Limit of detection: 20 ppb

KEY WORDS

SPE

REFERENCE

Thapar,S.; Bhushan,R.; Mathur,R.P. Degradation of organophosphorus and carbamate pesticides in soils -HPLC determination, *Biomed.Chromatogr.*, **1995**, *9*, 18–22.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb octyl
Mobile phase: MeCN:water 42:58 containing 100 mM n-butylamine, pH adjusted to 3.0 with perchloric acid
Flow rate: 1
Detector: UV 313

CHROMATOGRAM

Retention time: 11.40

OTHER SUBSTANCES

Simultaneous: oxantel, pyrantel

KEY WORDS

protect from light

REFERENCE

Allender,W.J. High-performance liquid chromatographic determination of oxantel and pyrantel pamoate, *J.Chromatogr.Sci.*, **1988**, *26*, 470–472.

SAMPLE

Matrix: solutions

Sample preparation: Pass 100 mL water through column A at 5 mL/min then elute the contents of column A onto column B with the mobile phase, elute column B with the mobile phase and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 4.6 5 μm Spherisorb ODS C18; B 250 × 4.6 5 μm Supelcosil LC-8 C8
Mobile phase: Gradient. MeCN:water 30:70 for 5 min, to 60:40 over 10 min, maintain at 60:40 for 10 min, to 30:70 over 5 min, maintain at 30:70 for 5 min and inject next sample.
Flow rate: 1.5
Injection volume: 100000
Detector: UV 220

CHROMATOGRAM
Retention time: 17.35
Limit of detection: 10 pg/mL

OTHER SUBSTANCES
Simultaneous: propoxur, carbofuran, captan, propham, chloropropham, barban, butylate

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KEY WORDS
water; drinking water; column-switching

REFERENCE
Marvin,C.H.; Brindle,I.D.; Hall,C.D.; Chiba,M. Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water, *J.Chromatogr.*, **1990**, *503*, 167–176.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 2 octadecylsilyl (Hewlett-Packard 79916 OD-552)
Mobile phase: MeOH:water 60:40
Column temperature: 25
Flow rate: 1.5
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: atrazine, DPX-4189, folpet, linuron

REFERENCE
Wang,Q-S.; Gao,R.-Y.; Wang,H.-Y. Computer-assisted optimization of selectivity (mobile phase, pH, and ion concentration) in high-performance liquid chromatography, *J.High Res.Chromatogr.*, **1990**, *13*, 173–177.

SAMPLE
Matrix: solutions
Sample preparation: Equilibrate column A with 10 mL MeCN and 10 mL water (pH 7). Pump 200 mL drinking water through column A at 3 mL/min, back flush contents of column A onto column B with the mobile phase and start the gradient.

HPLC VARIABLES

Column: A 10 × 2.1 5 μm RP-18 octadecylsilica (E. Merck); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:water from 40:60 to 60:40 over 15 min
Injection volume: 200000
Detector: UV 254

CHROMATOGRAM

Retention time: 8.7
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Extracted: azinphos-methyl, phosmet, parathion-methyl, azinphos-ethyl, fenitrothion, parathion, diazinon

KEY WORDS

drinking water; column-switching

REFERENCE

Driss,M.R.; Hennion,M.-C.; Bouguerra,M.L. Determination of carbaryl and some organophosphorus pesticides in drinking water using on-line liquid chromatographic preconcentration techniques, *J.Chromatogr.*, **1993**, *639*, 352–358.

SAMPLE

Matrix: solutions
Sample preparation: Condition a 10 × 4 55 mg 40 μm C18/OH Bondesil SPE cartridge (Varian/Analytichem) with 1 mL MeOH and 1 mL water, pass through 5 mL test water at 1 mL/min, pass through 500 μL pure water, elute the contents of the SPE cartridge onto the analytical column with mobile phase.

HPLC VARIABLES

Guard column: 10 × 4 4 μm Supersphere RP-8 (Merck)
Column: 250 × 4 4 μm Supersphere RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:water 20:80 containing 2.5 mM sodium acetate. B was MeOH:water 20:80 containing 2.5 mM sodium acetate. C was MeCN:water 60:40 containing 2.5 mM sodium acetate. A:B:C 75:25:0 for 5 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 5 min, re-equilibrate at initial conditions for 15 min.
Column temperature: 35
Flow rate: 0.75
Injection volume: 100
Detector: F ex 340 em 445 following post-column reaction. The column effluent flowed through a 50 × 4 Aminex A-27 (Bio-Rad) column at 120-140° and was mixed with reagent pumped at 1 mL/min, this mixture flowed through a 200 × 0.12 PTFE tube to the detector. (Reagent was prepared by adding 2 mL 25 mg/mL o-phthalaldehyde in MeCN and 100 μL 2-mercaptoethanol to 200 mL 5 mg/mL disodium tetraborate in water then making up to 250 mL with water.)

CHROMATOGRAM

Retention time: 24.53
Internal standard: trimethacarb (26.12)
Limit of detection: 0.03-0.05 ng/mL

OTHER SUBSTANCES

Simultaneous: aldicarb, bendiocarb, bufencarb, butocarboxim, carbanolate, carbofuran, cloethocarb, dioxacarb, ethiofencarb, fenobucarb, isoprocarb, methiocarb, methomyl, oxamyl, promecarb, propoxur, thiofanox, tranid

KEY WORDS
water; SPE; post-column reaction

REFERENCE
Hiemstra,M.; de Kok,A. Determination of N-methylcarbamate pesticides in environmental water samples using automated on-line trace enrichment with exchangeable cartridges and high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *667*, 155–166.

SAMPLE
Matrix: solutions
Sample preparation: Flush column A with 5 mL MeOH and 5 mL MeOH:pH 5.0 ammonium acetate, pass a 100 mL sample through the column at 4 mL/min, backflush the contents of column A onto column B and start the gradient, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 2 15-25 μm PLRP-S styrene-divinylbenzene co-polymer (Spark Holland); B 200 × 4 5 μm Spherisorb ODS2
Mobile phase: MeOH:100 mM pH 5.0 ammonium acetate from 30:70 to 88:12 over 34 min.
Column temperature: 40
Flow rate: 0.4
Injection volume: 100000
Detector: UV 280 or MS, Hewlett-Packard 5989 A, dual EI/chemical ionization source, ion source block 250°, quadrupole 100°, m/z 64-400, desolvation chamber 65°, helium nebulizer 50 psi, second-stage momentum separator 0.5 Torr, ion source chamber 15 μTorr

CHROMATOGRAM
Retention time: 22
Limit of detection: <1 ng/mL

OTHER SUBSTANCES
Simultaneous: propoxur, aldicarb, atrazine, barban, carbofuran, cyanazine, diuron, fluometuron, linuron, methomyl, monuron, oxamyl, simazine

KEY WORDS
water; column-switching

REFERENCE
Marcé,R.M.; Prosen,H.; Crespo,C.; Calull,M.; Borrull,F.; Brinkman,U.A.T. On-line trace enrichment of polar pesticides in environmental waters by reversed-phase liquid chromatography-diode array detection-particle beam mass spectrometry, *J.Chromatogr.A*, **1995**, *696*, 63–74.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 5 μm Kromasil-100-C18 (Akzo Nobel)
Column: 150 × 4 5 μm Kromasil-100-C18 (Akzo Nobel)
Mobile phase: MeCN:buffer 28:72 (Buffer was 820 mg/L sodium tetraborate decahydrate containing 50 μg/mL phthalaldehyde and 0.06 μL/mL 2-mercaptoethanol, adjusted to pH 8.5 with 100 mM HCl.)
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460 following post-column reaction. The column effluent flowed through a 3 m × 0.51 mm ID stainless steel tube at 140° to the detector. (Although the reagents are in the mobile phase the derivatization reaction does not take place until the

post-column reactor where the insecticides are hydrolyzed to methylamine that is then derivatized. This procedure avoids the use of a second pump for the post-column reagent.)

CHROMATOGRAM

Retention time: 19
Limit of detection: 700 pg

OTHER SUBSTANCES

Simultaneous: aldicarb, butocarboxim, carbofuran, dioxacarb, methomyl, propoxur

KEY WORDS

post-column reaction

REFERENCE

Sabala,A.; Portillo,J.L.; Broto-Puig,F.; Comellas,L. Development of a new high-performance liquid chromatography method to analyse N-methylcarbamate insecticides by a simple post-column derivatization system and fluorescence detection, *J.Chromatogr.A*, **1997**, *778*, 103–110.

SAMPLE

Matrix: tissue
Sample preparation: 21 g Liver + 60 g anhydrous sodium sulfate, mix with spatula, add 200 mL dichloromethane, mix with spatula, homogenize (VirTis 45) for 2 min at medium speed, filter through 5 g anhydrous sodium sulfate, re-extract tissue and sodium sulfate with 100 mL dichloromethane, filter, wash out flask with 25 mL dichloromethane, filter. Combine filtrates and filter them through 2 g anhydrous sodium sulfate, rinse flask with 20 mL dichloromethane, wash filter with 10 mL dichloromethane. Concentrate filtrate to 1-2 mL under reduced pressure at 30° (do not allow to go dry), transfer residue to a tube with 1-2 mL cyclohexane, wash in with dichloromethane:cyclohexane 50:50, make volume in tube 7.5 mL, filter (0.45 μm), add 5 mL to a 600 × 25 tube containing 60 g 200-400 mesh BioBeads SX-3 resin (Analytical BioChemistry Laboratories), pump through at 5 mL/min with dichloromethane:cyclohexane 50:50 mobile phase, collect fraction containing the compound, evaporate under reduced pressure at 30° to about 1 mL, make up to 2 mL with dichloromethane, add 1 mL to 1 mL 100 mg Bond Elut aminopropyl SPE cartridge (previously conditioned with 1 mL dichloromethane), elute with 3-5 mL dichloromethane: MeOH 98.5:1.5, evaporate eluate to dryness at 30° under reduced pressure (do not over dry), reconstitute in 200 μL MeOH, vortex for 5 s, filter (0.45 μm), inject a 20-30 μL aliquot.

HPLC VARIABLES

Guard column: Guard-PAK (Waters no. 88070)
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 12:88 to 70:30 over 30 min, to 80:20 over 1 min, maintain at 80:20 for 8 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1.5
Injection volume: 20-30
Detector: F ex 340 em 418, following post-column derivatization. The column effluent is mixed with 50 mM NaOH at 0.27 mL/min and the mixture flows through a 1 mL coil at 80° and is mixed with 140 μg/mL o-phthalaldehyde and 1 mL/L mercaptoethanol in 50 mM pH 10.5 potassium borate buffer pumped at 0.27 mL/min. The mixture flows through a 1 mL coil at 40° to the detector.

CHROMATOGRAM

Retention time: 23.1
Limit of quantitation: 5 ppb

OTHER SUBSTANCES

Extracted: methomyl, aldicarb, carbofuran, methiocarb, bufencarb

KEY WORDS
liver; pig; cow; duck; SPE; post-column reaction; derivatization

REFERENCE
Ali,M.S. Determination of *N*-methylcarbamate pesticides in liver by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 586–592.

SAMPLE
Matrix: water
Sample preparation: Extract 500 mL water with two 25 mL portions of dichloromethane, combine the extracts and dry them over anhydrous sodium sulfate for 10 min, evaporate to dryness under a stream of air, reconstitute with 40 μL acetone, add 300 μL 100 mM sodium carbonate, heat at 45-50° for 30-40 min, cool, add 300 μL acetone, add 100 μL 0.2% dansyl chloride in acetone, mix well, heat at 45° for 20 min, cool, evaporate the acetone under a stream of air, extract with 300 μL benzene (Caution! Benzene is a carcinogen!). Remove the organic layer and dry it over anhydrous sodium sulfate, inject a 1-10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.4 Zipax coated with 0.5% β,β'-oxydipropionitrile
Mobile phase: Hexane:EtOH 95:5
Injection volume: 1-10
Detector: F primary filter Turner 810, secondary filter Turner 827

CHROMATOGRAM
Retention time: k' 0.59

OTHER SUBSTANCES
Simultaneous: aldicarb (Temik), carbofuran, Carzol, dimethylamine, methomyl, methylamine, Mobam, propoxur (Baygon)

KEY WORDS
lake water; derivatization; normal phase

REFERENCE
Frei,R.W.; Lawrence,J.F.; Hope,J.; Cassidy,R.M. Analysis of carbamate insecticides by fluorigenic labelling and high-speed liquid chromatography, *J.Chromatogr.Sci.*, **1974**, *12*, 40–44.

SAMPLE
Matrix: water
Sample preparation: Condition a 4.6 mm dia C18 Empore SPE extraction disk with 10 mL MeOH at 1 mL/min and 10 mL water at 1 mL/min. Pass 10 mL water through the disk, backflush the contents of the disk on to the column with mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 4 μm Supersphere 60 RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:MeOH:water 40:40:20. B was MeCN:water 10:90. A:B from 5:95 to 20:80 over 15 min, to 30:70 over 20 min, to 65:35 over 20 min, to 100:0 over 7 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 0.8
Detector: F ex 330 em 465 following post-column reaction (LC.GC 1988, 6, 994) using thiofluor instead of 2-mercaptoethanol

CHROMATOGRAM
Retention time: 8 (aldicarb sulfoxide), 12 (aldicarb sulfone), 22.5 (3-hydroxycarbofuran), 37 (aldicarb), 40 (3-ketocarbofuran), 50 (carbofuran), 52.5 (carbaryl), 54 (1-naphthol)
Limit of detection: 5-40 pg/mL

OTHER SUBSTANCES
Extracted: aldicarb sulfoxide, aldicarb sulfone, aldicarb sulfoxide, carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, 1-naphthol

KEY WORDS
derivatization; SPE; post-column reaction

REFERENCE
Chiron,S.; Barceló,D. Determination of pesticides in drinking water by on-line solid-phase disk extraction followed by various liquid chromatographic systems, *J.Chromatogr.*, **1993**, *645*, 125–134.

SAMPLE
Matrix: water
Sample preparation: Filter, inject a 400 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3 μm HS-3C18 (Perkin Elmer)
Mobile phase: Gradient. MeCN:water from 5:95 to 20:80 over 13 min, to 65:35 over 15 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 8 min.
Flow rate: 1
Injection volume: 400
Detector: F ex 340 em 460 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1 mL/min and the mixture flowed through a 500 μL reaction coil at 95° to the detector. (Prepare the reagent by adding 1.25 mL 10 M NaOH to 100 mL water, add 10 mL 18 mg/mL N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor; Pickering Laboratories, Mountain Vie CA), add 2.5 mL 10 mg/mL o-phthalaldehyde in MeOH, make up to 250 mL with water, filter (0.45 μm nylon), degas with helium for 10 min before use. Prepare fresh each day.)

CHROMATOGRAM
Retention time: 29.78
Internal standard: 4-bromo-3,5-dimethylphenyl N-methylcarbamate (34)
Limit of detection: 0.4 ng/mL

OTHER SUBSTANCES
Simultaneous: aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, oxamyl, propoxur

KEY WORDS
post-column reaction

REFERENCE
Simon,V.A.; Pearson,K.S.; Taylor,A. Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high performance liquid chromatography with the use of fluorescence detection and a single o-phthalaldehyde post-column reaction, *J.Chromatogr.*, **1993**, *643*, 317–320.

SAMPLE
Matrix: water
Sample preparation: Prepare an SPE column by placing 500 mg 37-55 μm Bondapak octadecylsilica bonded-phase material in a 100 × 10 glass column fitted with a 40-100 μm glass fritted disc. Condition the SPE column with 5 mL MeOH and 10 mL water, do not allow to go dry. Add NaCl to the water sample so as to achieve a concentration of 10%, pass a 250 mL aliquot through the SPE column, elute with 10 mL MeCN:dichloromethane 50:50, evaporate the eluate at 60°, make up to 200 μL with MeCN, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 reversed-phase (Phase Sep)
Column: 250 × 4 Spherisorb C18
Mobile phase: Gradient. MeCN:water 23:77 for 0.1 min, to 45:55 over 14.9 min, to 70:30 over 25 min, re-equilibrate at initial conditions for 10 min.
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.09 ng/mL

OTHER SUBSTANCES

Simultaneous: carbendazim, carbofuran, dietofencarb, dioxacarb, fenothiocarb, iprodione, methomyl, methylthiofanate, molinate, oxamyl, thiobencarb

KEY WORDS

SPE; surface water

REFERENCE

Jiménez,B.; Moltó,J.C.; Font,G. Influence of dissolved humic material and ionic strength on C8 extraction of pesticides from water, *Chromatographia*, **1995**, *41*, 318–324.

SAMPLE

Matrix: water
Sample preparation: Mix water sample with 2 mL 500 mM NaOH, make up to 40 mL with water, shake for a few s, let stand for 10 min, add 1 mL glacial acetic acid, make up to 50 mL with water, filter (0.45 μm), sonicate the filtrate, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 33 × 4.6 3 μm Pecosphere 3x3 CR C18
Mobile phase: MeCN:water:glacial acetic acid 50:49.5:0.5 containing 10 mM sodium perchlorate
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, model 5021 conditioning cell 0 V, model 5011 dual analytical cell with porous graphite working electrodes at +0.1 V and +0.6 V (monitored), cells protected with 0.2 μm porous graphite filters

CHROMATOGRAM

Retention time: 1.21
Limit of detection: 0.98 nM

OTHER SUBSTANCES

Simultaneous: carbofuran, fenobucarb

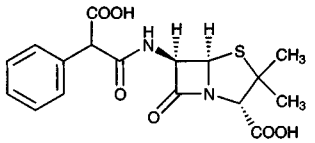
KEY WORDS

derivatization; river water

REFERENCE

Galeano Díaz,T.; Guiberteau,A.; Salinas,F.; Ortiz,J.M. Rapid and sensitive determination of carbaryl, carbofuran and fenobucarb by liquid chromatography with electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2681–2690.

Carbenicillin



Molecular formula: $C_{17}H_{18}N_2O_6S$
Molecular weight: 378.41
CAS Registry No.: 4697-36-3, 4800-94-6 (disodium salt), 17230-86-3 (potassium salt)
Merck Index: 1838
Lednicer No.: 1 414

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. 200 μL Serum + 200 μL 10 M urea, mix, filter (Amicon MPS-1 micropartition system with Amicon YMT membranes) while centrifuging at 1500 g for 10 min. Add 200 μL of the ultrafiltrate to 200 μL reagent and heat at 60° for 10 min, cool to room temperature, inject a 30-90 μL aliquot. Urine. Dilute urine 10-fold with water, filter (0.45 μm acrylate copolymer). Add 200 μL of the filtrate to 200 μL reagent and heat at 60° for 10 min, cool to room temperature, inject a 30-60 μL aliquot. (Prepare reagent by dissolving 13.81 g 1,2,4-triazole in 60 mL water, add 10 mL 2.7 mg/mL mercury(II) chloride in water, adjust pH to 9.0 ± 0.05 with saturated NaOH, make up to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Develosil ODS-5 (Nomura Chemicals)
Mobile phase: MeCN:buffer containing 5 mM tetrabutylammonium bromide and 5 mM sodium thiosulfate 1:1.8 (Prepare the buffer by dissolving 14.32 g $Na_2HPO_4.12H_2O$ and 6.240 g $NaH_2PO_4.2H_2O$ in 1 L water then diluting 100-fold.)
Column temperature: 40
Flow rate: 1
Injection volume: 30-90
Detector: UV 328

CHROMATOGRAM

Retention time: 4.5
Limit of detection: 1 μg/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES

Interfering: ticarcillin

KEY WORDS

serum; derivatization; ultrafiltrate

REFERENCE

Haginaka,J.; Wakai,J. High-performance liquid chromatographic assay of carbenicillin, ticarcillin and sulbenicillin in serum and urine using pre-column reaction with 1,2,4-triazole and mercury(II) chloride, *Analyst*, **1985**, *110*, 1185–1188.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a Bond Elut SAX SPE cartridge with 2 mL MeOH and 2 mL water. Plasma. Filter plasma (0.45 μm, Cosmonice, type W, Millipore), add 500 (human), 300 (rabbit) or 100 (rat) μL filtrate to ten volumes 50 mM ammonium acetate, add the mixture to the SPE cartridge, wash with 3 mL MeCN:500 mM acetic acid 50:50, wash with 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 500 μL MeOH:10% LiCl 40:60, inject a 20 μL aliquot. Urine. Dilute urine 40-fold with water, filter (0.45 μm, Cosmonice, type W, Millipore), add 500 μL filtrate to 5 mL 50 mM ammonium acetate, add the mixture to the SPE cartridge, wash with 3 mL MeCN:500 mM acetic acid 50:50,

wash with 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 1 mL MeOH:10% LiCl 40:60, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18-AR (Nacalia Tesque)
Mobile phase: MeOH:50 mM ammonium acetate 1:9 (human), 1:6 (rabbit), 1:7 (rat)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 18 (R), 24 (S)
Limit of detection: 10000 ng/mL

KEY WORDS
plasma; chiral; rat; rabbit; human; SPE

REFERENCE
Ishida,M.; Tsuda,Y.; Onuki,Y.; Itoh,T.; Shimada,H.; Yamada,H. Determination of carbenicillin epimers in plasma and urine with high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *652*, 43–49.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: 10 mM ammonium acetate
Flow rate: 1.6
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 5, 6 (two isomers)

OTHER SUBSTANCES
Interfering: ticarcillin

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE
Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil C18-AR (Nacalai Tesque Co., Kyoto)
Mobile phase: MeOH:50 mM pH 7.0 10:80 or MeOH:50 mM ammonium acetate 10:70
Flow rate: 0.9
Injection volume: 20-40
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: sulbenicillin

KEY WORDS
carbenicillin is IS

REFERENCE
Itoh,T.; Watanabe,N.; Ishida,M.; Tsuda,Y.; Koyano,S.; Tsunoi,T.; Shimada,H.; Yamada,H. Stereoselective disposition of sulbenicillin in humans, *Antimicrob.Agents Chemother.*, **1998**, *42*, 325–331.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: dicloxacillin, methicillin, penicillin G, penicillin V, cloxacillin, nafcillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10

Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 13.7

OTHER SUBSTANCES

Simultaneous: cephapirin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

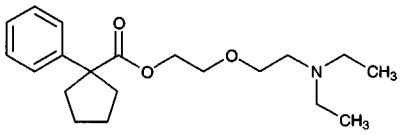
KEY WORDS

derivatization

REFERENCE

Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

Carbetapentane



Molecular formula: $C_{20}H_{31}NO_3$
Molecular weight: 333.47
CAS Registry No.: 77-23-6, 23142-01-0 (citrate)
Merck Index: 1840

SAMPLE

Matrix: formulations
Sample preparation: Mix 2-4 mL syrup or expectorant oral solution with 4 mL 10 μg/mL IS in MeOH. Make up to 25 mL with MeOH. Inject a 20 μL. aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm LiChrosphere 100RP-18
Mobile phase: MeOH:25% (w/w) ammonia 99.2:0.8
Flow rate: 1.2
Injection volume: 20
Detector: UV 258

CHROMATOGRAM

Retention time: k' 1.95
Internal standard: chlorpromazine (k' 2.78)
Limit of detection: 8 μg/mL

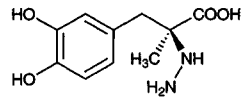
KEY WORDS

syrup; oral solution

REFERENCE

Gad-Kariem,E.A.; Abounassif,M.A. Determination of pentoxyverine in cough preparations by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3049–3059.

Carbidopa



Molecular formula: $C_{10}H_{14}N_2O_4$
Molecular weight: 226.23
CAS Registry No.: 28860-95-9, 38821-49-7 (monohydrate)
Merck Index: 1843
Lednicer No.: 2 119

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 μL 4 M perchloric acid + 50 μL 1 μg/mL dihydroxybenzylamine in 0.1 M perchloric acid, centrifuge at 1500 g for 10 min. Remove 300 μL supernatant and centrifuge it at 1600 g through a 0.2 μm regenerated cellulose filter, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Biophase ODS + 50 × 4.6 Pelliguard LC-18
Column: 250 × 4.6 5 μm Biophase ODS or 250 × 4.6 Phase II ODS (both from Bioanalytical Systems)
Mobile phase: MeOH:buffer 5:95 (Buffer was 20 mM sodium citrate, 100 mM NaH_2PO_4, 0.15 mM, and 1.25 mM heptanesulfonic acid, pH 3.2.)
Column temperature: 28
Flow rate: 1-1.5
Injection volume: 20
Detector: E, Bioanalytical Systems LC-150 in dual-parallel mode, channel 1 700 mV 200 nA f.s. for levodopa and 3-O-methyldopa, channel 2 560 mV 10 nA f.s. for dopamine, carbidopa, and dihydroxyphenylacetic acid, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.1
Internal standard: dihydroxybenzylamine (7)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES

Simultaneous: 3-O-methyldopa, dopamine, levodopa, dihydroxyphenylacetic acid

KEY WORDS

plasma

REFERENCE

Cedarbaum,J.M.; Williamson,R.; Kutt,H. Simultaneous determination of levodopa, its metabolites and carbidopa in clinical samples, *J.Chromatogr.*, **1987**, *415*, 393–399.

SAMPLE

Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex pre-swollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. Add 1 mL plasma to column, elute with 5.5 mL water, discard first 1 mL. Add next 4.5 mL to 0.5 mL 0.5 M perchloric acid, centrifuge, inject 10 μL aliquot of supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid

Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: O-methyldopa, levodopa, dihydroxyphenylacetic acid (DOPAC)

KEY WORDS
plasma

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 100 μL 4 M perchloric acid, vortex, centrifuge at 2000 g for 10 min, inject a 60 μL aliquot of the supernatant (keep sample tray at 6 ± 1°).

HPLC VARIABLES
Guard column: 45 × 4.6 37-40 μm Whatman pellicular-ODS followed by 45 × 4.6 5 μm Ultrasphere-IP C18
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:20 mM orthophosphoric acid and 4 mM sodium octanesulfonate 25:75 adjusted to pH 2.8 ± 0.05 with 50% NaOH
Column temperature: 40
Flow rate: 1
Injection volume: 60
Detector: E, BAS LC-4B, 0.75 V vs Ag/AgCl, 5 nA full scale for carbidopa, 20 nA full scale for 3-O-methyldopa and levodopa

CHROMATOGRAM
Retention time: 10.9
Internal standard: α-ethyldopa (15.4)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: levodopa, 3-O-methyldopa

KEY WORDS
plasma; stabilize plasma sample immediately with EDTA and 2 mg/mL sodium metabisulfite

REFERENCE
Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

SAMPLE
Matrix: blood

Sample preparation: 4 mL Plasma + 500 μL 20 mg/mL ascorbic acid solution, vortex for 30 s. 1 mL Aliquot + 75 mg acid washed alumina + 100 μL 1 μg/mL 3,4-dihydroxybenzylamine hydrobromide in buffer, vortex, add 1 mL 1.5 M pH 8.6 TRIS buffer, shake at 230 oscillations/min for 15 min. Allow to settle and discard plasma, wash the alumina twice by shaking with 5 mL water for 10 min. To the washed alumina add 900 μL buffer, vortex for 20 s, allow to settle, inject a 50 μL aliquot of the supernatant. (Buffer was 200 mM phosphoric acid containing 3.3 μm EDTA and 6.7 μm potassium metabisulfite.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:buffer 8:92 (Buffer was pH 2.6 550 mM NaH_2PO_4 containing 1 mM sodium octyl sulfate and 0.7 mM EDTA.)
Flow rate: 1.5
Injection volume: 50
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode, Ag/AgCl reference electrode, 0.75 V.

CHROMATOGRAM

Retention time: 9.7
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (4.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: levodopa
Noninterfering: caffeine, ibuprofen, aspirin, nicotine, acetaminophen, theophylline

KEY WORDS

plasma; SPE

REFERENCE

Miller,R.B.; Dehelean,L.; Bélanger,L. Determination of carbidopa and levodopa in human plasma by high-performance liquid chromatography with electrochemical detection, *Chromatographia*, **1993**, *35*, 607–612.

SAMPLE

Matrix: formulations
Sample preparation: Powder levodopa/carbidopa tablets or contents of capsules, weigh out an amount equivalent to about 100 mg levodopa, add 30 mL 0.1 M HCl, sonicate, make up to 50 mL with 0.1 M HCl, mix, filter (0.45 μm), discard first 5 mL filtrate. 10 mL Filtrate + 50 mL 0.5 mg/mL acetaminophen in MeOH:mobile phase 75:175, make up to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 3% aqueous acetic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 5
Internal standard: acetaminophen (9)

OTHER SUBSTANCES

Simultaneous: levodopa

KEY WORDS

capsules; tablets

REFERENCE
Ting,S. Liquid chromatographic determination of levodopa and levodopa-carbidopa in solid dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 987–990.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:50 mM ammonium acetate adjusted to pH 4.1 with 0.6 M acetic acid 1:99
Flow rate: 0.9
Detector: E, Coulochem model 5100A, screen electrode +0.3 V, sample electrode +0.6 V and UV 280

CHROMATOGRAM
Retention time: 11.4
Limit of detection: 200 ng/mL (UV), 2 ng/mL (E)

OTHER SUBSTANCES
Simultaneous: hydroxydopa, levodopa, methyldopa, methoxytyrosine, methylcarbidopa, impurities

KEY WORDS
stability-indicating; tablets

REFERENCE
Kafil,J.B.; Dhingra,B.S. Stability-indicating method for the determination of levodopa, levodopa-carbidopa and related impurities, *J.Chromatogr.A*, **1994**, *667*, 175–181.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: dopamine, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, norepinephrine, phenylephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE

Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE

Matrix: urine

Sample preparation: 100 μL Urine + 100 μL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA + 25 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 25 mg alumina + 1 mL 2 M pH 8.6 Tris-HCl buffer in a microfilter tube (Centrex, Schleicher & Schuell), vortex 5 min, allow to stand for 10 min, filter off water, wash with 5 mL water, add 5 mL water, centrifuge at 3000 g, vortex with 400 μL 0.2 M perchloric acid containing 11 mM disodium EDTA and 0.4 M sodium metabisulfite, centrifuge at 9000 g for 5 min, inject 50 μL of filtrate.

HPLC VARIABLES

Guard column: 40 × 4.6 Bio-Sil ODS-10 (Bio-Rad)

Column: 250 × 4.6 5 μm Ultrasphere IP C18

Mobile phase: MeOH:water 22.5:77.5 containing 20 mM citric acid, 20 mM Na_2HPO_4, 4 mM sodium octanesulfonate, and 0.05 mM disodium EDTA, pH adjusted to 2.74 ± 0.01 with 2 M citric acid

Column temperature: 40

Injection volume: 50

Detector: E, BAS LC-4B, 0.54 V vs Ag/AgCl, 50 nA full scale

CHROMATOGRAM

Retention time: 10

Internal standard: α-ethyldopa (14)

Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Extracted: levodopa, dopamine

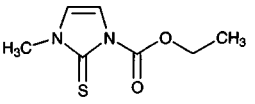
KEY WORDS

stabilize each 10 mL urine sample immediately with 0.5 mL 0.1 M HCl and 1 mL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA; SPE

REFERENCE

Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

Carbimazole



Molecular formula: $C_7H_{10}N_2O_2S$
Molecular weight: 186.23
CAS Registry No.: 22232-54-8
Merck Index: 1844
Lednicer No.: 1 240

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.138

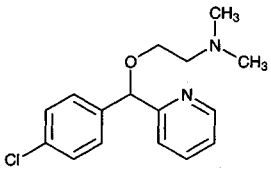
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Carbinoxamine



Molecular formula: $C_{16}H_{19}ClN_2O$
Molecular weight: 290.79
CAS Registry No.: 486-16-8, 3505-38-2 (maleate), 49746-00-1 (l-form tartrate)
Merck Index: 1845
Lednicer No.: 1 43

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 30 μL 1 μg/mL phenyltoloxamine in water + 200 μL ammonia, extract twice with 7 mL pentane:diethyl ether 75:25. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 150 μL mobile phase, inject a 90 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb CN cyanopropyl
Mobile phase: MeCN:5 mM pH 6 phosphate buffer 40:60
Flow rate: 1
Injection volume: 90
Detector: E, Environmental Science Associates Coulochem model 5010, screen mode +0.55 V and +0.90 V

CHROMATOGRAM

Retention time: 15
Internal standard: phenyltoloxamine (19)
Limit of detection: 0.5 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Stockis,A.; Deroubaix,X.; Jeanbaptiste,B.; Lins,R.; Allemon,A.M.; Laufen,H. Relative bioavailability of carbinoxamine and phenylephrine from a retard capsule after single and repeated dose administration in healthy subjects, *Arzneimittelforschung*, **1995**, *45*, 1009–1012.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 226

CHROMATOGRAM

Retention time: 5.44
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.81

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.98

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.98 (of first (+) enantiomer)

KEY WORDS
chiral; α 1.31

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chiracel OD
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5.25, 6.25 (enantiomers)

KEY WORDS
chiral

REFERENCE
Baxter Scientific Products Catalog, 1992-3, p. 213, p. 213.

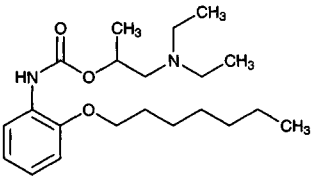
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 40:50:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 262

KEY WORDS
chiral; α = 1.15 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Carbisocaine



Molecular formula: $C_{21}H_{36}N_2O_3$
Molecular weight: 364.52
CAS Registry No.: 76629-87-3, 68931-03-3 (HCl)

SAMPLE
Matrix: bulk

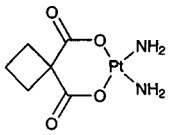
HPLC VARIABLES
Column: 250 × 4 5 μm LiChroCART ChiraDex (Merck)
Mobile phase: MeCN:0.03% pH 6.1 triethylamine 5:95
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 26 (R-(-)), 28 (S-(+))

KEY WORDS
chiral

REFERENCE
Cizmárik,J.; Lehotay,J.; Hromul'áková,K.; Pokorná,M.; Lacuska,M. HPLC separation of enantiomers of carbisocaine. Study of local anaesthetics, part 138, *Pharmazie*, **1997**, *52*, 402–403.

Carboplatin



Molecular formula: $C_6H_{12}N_2O_4Pt$
Molecular weight: 371.25
CAS Registry No.: 41575-94-4
Merck Index: 1870
Lednicer No.: 4 16

SAMPLE

Matrix: blood
Sample preparation: Inject an aliquot of plasma ultrafiltrate directly (Further sample clean up not required when using post-column reaction detection.).

HPLC VARIABLES

Column: 150 × 4.6 3 μm YMC ODS-AQ
Mobile phase: 20 mM NaH_2PO_4
Flow rate: 0.7
Detector: UV 290 following post-column reaction detection. The column effluent mixed with 20 mM pH 5.4 NaH_2PO_4 containing 40 mM sodium bisulfite pumped at 0.7 mL/min and the mixture flowed through a 15.2 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Limit of detection: 13 ng/mL
Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; ultrafiltrate; post-column reaction

REFERENCE

Burns,R.B.; Embree,L. Comparison of ultraviolet (UV) and post-column reaction (PC/UV) detection methods for the high-performance liquid chromatographic (HPLC) analysis of carboplatin in human plasma ultrafiltrate (PUF) (Abstract 2483), *Pharm.Res.*, **1997**, *14*, S373–S373.

SAMPLE

Matrix: formulations
Sample preparation: Dilute carboplatin injections with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak ODS
Mobile phase: Gradient. A was MeOH. B was 0.02% aqueous formic acid. A:B 0:100 for 6 min, from 0:100 to 25:75 in 1 min, maintain at 25:75 for 8 min
Flow rate: 0.5
Injection volume: 20
Detector: MS, Fisons VG Quattro Quadropole, electrospray, source 100°, cone voltage 30 V, scan m/z 200-1000, m/z 372

CHROMATOGRAM

Retention time: 5.3
Limit of detection: 35 ng/mL

OTHER SUBSTANCES

Simultaneous: DWA2114R

KEY WORDS

injections

REFERENCE
Burns,R.B.; Burton,R.W.; Albon,S.P.; Embree,L. Liquid chromatography-mass spectrometry for the detection of platinum antineoplastic complexes, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 367–372.

SAMPLE
Matrix: formulations
Sample preparation: Dilute carboplatin injections with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak ODS
Mobile phase: MeCN:0.02% aqueous formic acid 2:98
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 200 ng/mL

KEY WORDS
injections

REFERENCE
Burns,R.B.; Burton,R.W.; Albon,S.P.; Embree,L. Liquid chromatography-mass spectrometry for the detection of platinum antineoplastic complexes, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 367–372.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: water
Flow rate: 2.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 3.10

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 3 μm YMC ODS-AQ
Mobile phase: MeCN:20 mM NaH_2PO_4 1.3:98.7
Flow rate: 0.7
Detector: UV 230

CHROMATOGRAM

Limit of detection: 25 ng/mL
Limit of quantitation: 75 ng/mL

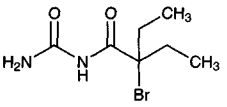
KEY WORDS

comparison with post-column reaction detection

REFERENCE

Burns,R.B.; Embree,L. Comparison of ultraviolet (UV) and post-column reaction (PC/UV) detection methods for the high-performance liquid chromatographic (HPLC) analysis of carboplatin in human plasma ultrafiltrate (PUF) (Abstract 2483), *Pharm.Res.*, **1997**, *14*, S373–S373.

Carbromal



Molecular formula: $C_7H_{13}BrN_2O_2$
Molecular weight: 237.10
CAS Registry No.: 77-65-6
Merck Index: 1879

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

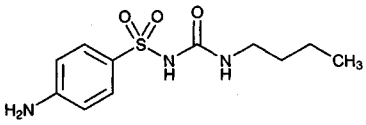
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben,

pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Carbutamide



Molecular formula: $C_{11}H_{17}N_3O_3S$
Molecular weight: 271.34
CAS Registry No.: 339-43-5
Merck Index: 1881
Lednicer No.: 1 138

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.547

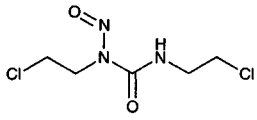
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Carmustine



Molecular formula: $C_5H_9Cl_2N_3O_2$
Molecular weight: 214.05
CAS Registry No.: 154-93-8
Merck Index: 1894
Lednicer No.: 2 12

SAMPLE

Matrix: blood
Sample preparation: 2-5 mL Whole blood or plasma + 32 mg sodium ascorbate + 200 mg phenytoin + 2-5 mL MeCN, shake or vortex for about 5 min, centrifuge at 2000 rpm for 5-10 min, filter (Millipore sample filtration kit), inject an aliquot of the filtrate

HPLC VARIABLES

Guard column: Altex
Column: 150 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeOH:water 50:50
Flow rate: 1
Detector: UV 237

CHROMATOGRAM

Retention time: 6
Internal standard: phenytoin (9)
Limit of detection: 100 ng/mL

KEY WORDS

whole blood; plasma; handle under yellow light

REFERENCE

Krull,I.S.; Strauss,J.; Hochberg,F.; Zervas,N.T. An improved trace analysis for N-nitrosoureas from biological media, *J.Anal.Toxicol.*, **1981**, *5*, 42–46.

SAMPLE

Matrix: blood
Sample preparation: Acidify plasma by adding 4% (v/v) glacial acetic acid to a final pH of 4. 500 μL Plasma + 1.68 μg propyl paraben in EtOH:water 5:95, vortex for 5 s, add 4 mL diethyl ether:EtOH 98.75:1.25, shake mechanically for 15 min, centrifuge at 2000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of air at 35°, reconstitute the residue in mobile phase, vortex for 10 s, inject an aliquot

HPLC VARIABLES

Guard column: 70 × 2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:0.1% acetic acid 35:65
Flow rate: 1.2
Detector: UV 230

CHROMATOGRAM

Retention time: 13.8
Internal standard: propyl paraben (16.9)
Limit of detection: 50 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE
Yeager,R.L.; Oldfield,E.H.; Chatterji,D.C. Quantitation of 1,3-bis(2-chloroethyl)-1-nitrosourea in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *305*, 496–501.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg CBA Bond Elut SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge blood at 5000 g for 2-3 min, freeze plasma in dry ice/hexane within 1 min. Thaw within 3 min by immersion in a 50° water bath. 1 mL Thawed plasma + 500 μL 2.5 μg/mL IS in 100 mM citric acid, vortex for 5 s, centrifuge for 5 min, add a 1 mL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, elute with 200 μL MeOH into a vial containing 50 μL 100 mM acetic acid, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM ammonium acetate 30:70 adjusted to pH 4.4 with glacial acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 8.5
Internal standard: 1-methyl-3-isobutyl-8-vinyl-2,6-dioxopurine (S10338) (7.2)

OTHER SUBSTANCES
Extracted: lomustine, fotemustine

KEY WORDS
plasma; SPE

REFERENCE
Gordon,B.H.; Richards,R.P.; Hiley,M.P.; Gray,A.J.; Ings,R.M.; Campbell,D.B. A new method for the measurement of nitrosoureas in plasma: an h.p.l.c. procedure for the measurement of fotemustine kinetics, *Xenobiotica*, **1989**, *19*, 329–339.

SAMPLE
Matrix: blood
Sample preparation: Add fotemustine in ethanol PBS to plasma. Mix 100 μL plasma with 600 μL diethyl ether, rotate, centrifuge. Remove 400 μL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 mm long 4 μm Novapack
Mobile phase: EtOH:1% acetic acid (pH 3) 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.6
Internal standard: fotemustine (7.6)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; rat

REFERENCE

Meulemans,A.; Giroux,B.; Hannoun,P.; Henzel,D.; Bizzari,J.P.; Mohler,J. Permeability of two nitrosoureas, carmustine and fotemustine in rat cortex, *Chemotherapy*, **1989**, *35*, 313–319.

SAMPLE

Matrix: reaction mixtures

Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ (pH 4.7) 55:45
Flow rate: 1
Injection volume: 20
Detector: UV 228

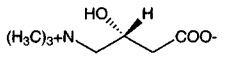
CHROMATOGRAM

Retention time: 8.0
Limit of detection: 160 ng/mL

REFERENCE

Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Carnitine



Molecular formula: $C_7H_{15}NO_3$
Molecular weight: 161.20
CAS Registry No.: 461-06-3, 541-15-1 (L-form)
Merck Index: 1898

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg LiChrolut SCX SPE cartridge (Merck) with 2 mL water. Condition a Toyopak IC-SP S SPE cartridge (Tosoh) with 2 mL water. 50 μL Plasma + 10 μL 400 μM IS in water + 1 mL 25 mM pH 1.0 sodium phosphate buffer, mix, add to the LiChrolut SCX SPE cartridge, wash with 5 mL water, elute with 1 mL isopropanol:150 mM pyridine in water 50:50. Evaporate the eluate under reduced pressure, add 20 μL 2.5 mM 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole in DMF, add 20 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in DMF, add 10 μL pyridine, vortex, let stand at room temperature for 1 h, add 1 mL DMF:water 40:60, add to the Toyopak IC-SP S SPE cartridge, wash with 1 mL DMF:water 40:60, wash with 10 mL water, wash with 100 μL 600 mM KCl in MeOH:water 50:50, elute with 200 μL 600 mM KCl in MeOH:water 50:50, inject an aliquot of the eluate. (Synthesis of the reagent, 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole, is as follows. Add 3.15 g benzil and 2.46 g methyl 4-formylbenzoate (terephthalaldehydic acid methyl ester) to 10 g ammonium acetate in 30 mL acetic acid, stir at 80° for 9 h, cool to room temperature, pour into cold water, filter. Wash the precipitate with water and recrystallize it from EtOH to give 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid methyl ester as pale yellow crystals (mp 245-248°). Reflux 1.47 g 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid methyl ester and 15 mL 80% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) in 100 mL EtOH for 4 h, cool, to room temperature, pour into cold water, filter. Wash the precipitate with water and recrystallize it from EtOH:benzene 50:50 (Caution! Benzene is a carcinogen!) to give 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole as a colorless powder (mp >300°) (J. Chromatogr. 1993, 619, 1).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Daisopak SP-120-ODS (Daiso, Osaka)
Mobile phase: Gradient. A was MeCN. B was MeCN:200 mM pH 7.0 Tris-HCl buffer 20:80. A:B 25:75 for 12 min, to 45:55 over 10 min, to 100:0 over 5 min, maintain at 100:0 for 10 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 475

CHROMATOGRAM

Retention time: 7.3
Internal standard: cyclohexanoylcarnitine (Synthesis of cyclohexanoylcarnitine is as follows. Dissolve 300 mg carnitine in 500 μL trifluoroacetic acid, add 1 mL cyclohexanecarbonyl chloride, protect from moisture with a calcium chloride tube, mix, heat at 40-45° overnight, cool to room temperature, add 5 mL acetone, cool on ice for a couple of h, centrifuge to remove undissolved material, add diethyl ether to incipient cloudiness, when crystallization starts add 10 mL diethyl ether, cool on ice. Dissolve the crystallization products in 1 mL MeOH, add 4-5 mL acetone, add diethyl ether to incipient cloudiness, when crystallization starts add 5 mL diethyl ether (cf. Biochim. Biophys. Acta 1968, 152, 559) to obtain cyclohexanoylcarnitine hydrochloride (mp 159-160°).) (20)
Limit of detection: 240 nM

OTHER SUBSTANCES

Extracted: acetylcarnitine, hexanoylcarnitine, octanoylcarnitine, propionylcarnitine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Kuroda,N.; Ohyama,Y.; Nakashima,K.; Akiyama,S. HPLC determination of carnitine and acylcarnitines in human plasma by means of fluorescence labeling using 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole, *Chem.Pharm.Bull.*, **1996**, *44*, 1525–1529.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg SAX-Isolute SPE cartridge (Stepbio, Bologna) with 500 μL MeOH and 1 mL water. 100 μL Plasma + 30 μL 17.6 μg/mL methanesulfonyl-L-carnitine in water containing 640 ng/mL isobutyryl-L-carnitine + 370 μL water, add to the SPE cartridge, elute with 500 μL 10 mM pH 3.5 phosphate buffer. With continuous vortexing add 20 μL 1 M HCl, 100 μL 16 mg/mL 1-aminoanthracene in acetone, and 100 μL 160 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in 10 mM pH 3.5 NaH_2PO_4 buffer (in 20 μL aliquots) to the eluate, let stand at 25° for 20 min, wash with 5 mL diethyl ether. Remove a 300 μL aliquot of the aqueous phase and add it to 700 μL 10 mM pH 9.1 Na_2HPO_4 buffer, wash with 5 mL chloroform. Remove a 500 μL aliquot of the aqueous phase and add it to 500 μL 10 mM pH 3.5 NaH_2PO_4 buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Kromasil C18
Mobile phase: MeCN:100 mM pH 3.5 ammonium acetate 30:70
Flow rate: 1.3
Injection volume: 20
Detector: F ex 248 em 418

CHROMATOGRAM
Retention time: 8
Internal standard: methanesulfonyl-L-carnitine (14.5), isobutryl-L-carnitine (21)
Limit of quantitation: 5 μM

OTHER SUBSTANCES
Extracted: acetylcarnitine, propionylcarnitine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Longo,A.; Bruno,G.; Curti,S.; Mancinelli,A.; Miotto,G. Determination of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma by high-performance liquid chromatography after pre-column derivatization with 1-aminoanthracene, *J.Chromatogr.B*, **1996**, *686*, 129–139.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Potter-Elvehjem) 200-300 mg tissue with 8 volumes cold 6% perchloric acid or mix plasma with two volumes of 6% perchloric acid, centrifuge at 5000 g for 10 min, wash pellet with 6% (tissue) or 3% (plasma) perchloric acid, centrifuge again, combine the supernatants, neutralize with KOH, allow to stand on ice for 30 min, centrifuge, filter (0.45 μm, Biofield chromatodisc 13 A). To a total volume of 1 mL add filtrate, 0.5 μmole EDTA, 10 μmole pH 7.5 phosphate buffer, 40 nmole acetylcoenzyme A, and 1 U carnitine acetyltransferase, incubate at 25° for 30 min, adjust pH to 2 with phosphoric acid, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Unisil QC8 (Gasukuro Kogyo)

Mobile phase: MeOH:190 mM KH_2PO_4 13:87
Flow rate: 0.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8 (of coenzyme A, the product of the reaction)

KEY WORDS
plasma; Guinea pig; liver; kidney; heart; muscle; enzymic reaction

REFERENCE
Arakawa,N.; Ha,T.Y.; Otsuka,M. An improved high-performance liquid chromatographic assay for the determination of free and esterified carnitine in animal tissues, *J.Nutr.Sci.Vitaminol.(Tokyo)*, **1989**, *35*, 475–479.

SAMPLE
Matrix: blood, urine
Sample preparation: Pack a disposable polypropylene chromatography column with 0.5 mL 230-400 mesh Silica gel 60 (Curtin Matheson). 25 μL Urine (or 10 μL urine + 20 μL water or 100 μL plasma) + 25 μL 100 μM IS + 25 μL 1 M KH_2PO_4 + 1 mL MeCN:MeOH 75:25, vortex for 2 s, centrifuge at 13600 g for 5 min, add the supernatant to the silica gel column, wash with 2 mL MeOH, wash with 1 mL 1% acetic acid in MeOH, elute with 4 mL 1% acetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 250 μL MeCN:MeOH 75:25, vortex for 2 s, centrifuge at 13600 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 10 μL diisopropylethylamine solution and 20 μL 100 mM 4'-bromophenacyl trifluoromethanesulfonate in MeCN, vortex for 2 s, let stand for 10 min, inject a 6 μL aliquot. (The diisopropylethylamine solution was 25 μL diisopropylethylamine in 10 mL MeOH. Prepare 4'-bromophenacyl trifluoromethanesulfonate as follows. Add 8.8 g p-bromobenzoyl chloride in 40 mL dry ether over 20-30 min to 100 mmoles diazomethane stirred in an ice bath, stir in an ice bath for 8-9 h, let stand at room temperature for 3 h, evaporate the solvent under reduced pressure, recrystallize 4'-bromo-2-diazoacetophenone from ether/hexane (mp 123.5-124° d) (J.Am.Chem.Soc. 1951, 73, 5301). Condense 50 mL anhydrous sulfur dioxide in a flask fitted with a calcium sulfate drying tube, cool in a dry ice/acetone bath, add 2.25 g 4'-bromo-2-diazoacetophenone, stir for 5 min, add 900 μL anhydrous trifluoromethanesulfonic acid from a freshly opened bottle in one portion, stir for 15 min, remove the cooling bath, after 30 min use an ice/water bath to evaporate the solvent. Dissolve the residue in 100 mL boiling dichloromethane, treat twice with 5 g portions of decolorizing carbon, filter, evaporate the filtrate, recrystallize the residue from pentane:dichloromethane 80:20 to give 4'-bromophenacyl trifluoromethanesulfonate as colorless plates (mp 137-8°) (J.Chromatogr. 1984, 299, 365).)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil (MOS-1) C8
Mobile phase: Gradient. A was MeCN:water 80:20. B was MeCN:water 20:80. C was MeCN:water:phosphoric acid:triethylamine 20:80:0.4:0.5. D was MeCN:water:phosphoric acid:triethylamine 90:10:0.2:0.25. A:B:C:D 100:0:0:0 for 0.2 min, 0:100:0:0 for 0.8 min, then 0:0:100:0 to 0:0:0:100 over 10 min, then 100:0:0:0 for 3 min before next run.
Flow rate: 1.75
Injection volume: 6
Detector: UV 260

CHROMATOGRAM
Retention time: 4.25
Internal standard: 4-(N,N-dimethyl-N-ethylammonio)-3-hydroxybutanoate (Prepare by N-demethylating carnitine and alkylating the resulting 4-(N,N-dimethylamino)-3-hydroxy-

butanoic acid with iodoethane. Perform the reaction under nitrogen and protect from light. Heat 83 g thiophenol and 20 g NaOH in 100 mL EtOH until they dissolve, add 700 mL toluene, distil slowly at atmospheric pressure, add 600 mL toluene in 100 mL portions to keep the volume in the flask at 500-600 mL, continue distillation for 1 h after the distillation head temperature reaches 115°. Sodium thiophenoxide crystallizes out in the flask as the distillation progresses (Anal.Chem. 1968, 40, 125). Filter the product under nitrogen and wash it with boiling toluene, store in the dark under vacuum. Dissolve 2 g l-carnitine chloride in 100 mL DMF with stirring at 80°, cool to room temperature, add 6.6 g sodium thiophenoxide, stir for 20 min, heat at 100° with stirring for 8 h, cool to room temperature, pour into 150 g ice and 4.5 mL concentrated HCl, wash 4 times with 100 mL portions of diethyl ether, evaporate the aqueous phase under reduced pressure at 40°, add more water to the flask until all the DMF is removed. When the volume has been reduced to 5 mL adjust pH to 10 with NaOH, add to a 300 × 20 column of 200-400 mesh Dowex 2X8 (OH^-) made up in 50 mM NaOH, wash with 200 mL 50 mM NaOH at 1.5 mL/min, wash with water until the pH of the effluent is neutral. Place the resin in a beaker containing ice water, slowly add concentrated HCl with stirring until the supernatant reaches pH 1, pour the resin into the column, wash the column with 200 mL 100 mM HCl. Collect all the effluents and concentrate them almost to dryness under reduced pressure, add to a 500 × 20 column of 200-400 mesh Dowex 50X8 (H^+ form), elute with 300 mL 500 mM HCl and 300 mL 2.5 M HCl, collect 10 mL fractions, identify fractions containing compound with iodoplatinate spray reagent. Combine fractions containing compound and lyophilize them to give 4-(N,N-dimethylamino)-3-hydroxybutanoic acid as a colorless glass. Dissolve 50 mg 4-(N,N-dimethylamino)-3-hydroxybutanoic acid and 150 mg barium hydroxide octahydrate in 1 mL water, add iodoethane in 5 mL MeOH, stir for 15 h, evaporate to dryness, reconstitute with 2 mL water, add 500 μL 1 M sulfuric acid, centrifuge, wash the solid with 100 mM sulfuric acid. Combine the aqueous layers and adjust the pH to 7 with KOH, add to a 190 × 12 column of 200-400 mesh Dowex 1X8 (OH^-), elute with water and collect fractions. Adjust the pH of the fractions containing the product to 4 with HCl, evaporate, add the residue to a 190 × 12 column of 200-400 mesh Dowex 50X8 (H^+) form, elute column with 60 mL 1 M HCl and 200 mL 2 M HCl, collect fractions (J.Labelled Cmpds.Radiopharm. 1982, 9, 535, J.Chromatogr. 1984, 336, 271, Clin.Chim.Acta 1992, 212, 55).) (4.5)

Limit of quantitation: 10 nmole/mL

OTHER SUBSTANCES

Extracted: carnitine esters, betaine, butyrobetaine, trimethyllysine

KEY WORDS

plasma; SPE; derivatization; plasma details (see Anal. Biochem. 1993; 212; 510)

REFERENCE

Minkler,P.E.; Hoppel,C.L. Quantification of carnitine and specific acylcarnitines by high-performance liquid chromatography: Application to normal human urine and urine from patients with methylmalonic aciduria, isovaleric acidemia or medium-chain acyl-CoA dehydrogenase deficiency, *J.Chromatogr.*, **1993**, *613*, 203–221.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 287.8

CHROMATOGRAM

Retention time: 16.058

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk
Sample preparation: React 50 μmoles carnitines, 2 mmoles 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole, and 35 mmoles 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in 100 μL pyridine:DMF 20:80, let stand at room temperature for 2 h, add 900 μL 10 mM HCl in MeOH:water 80:20, add to a 150 μL 0.06 mequiv Toyopak IC-SP S (Tosoh) SPE cartridge, wash with 5 mL 10 mM HCl:MeOH 50:50, elute with 3 mL 1 M pH 7.0 triethylamine acetate in MeOH. Evaporate the eluate to dryness, reconstitute with 1 mL 100 mM trifluoroacetic acid:DMF 20:80, inject an aliquot. (Preparation of 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole is as follows. Add 10 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 10 mL MeCN dropwise to a stirred solution of 550 μmoles ethylenediamine in MeCN over 30 min, stir at room temperature for 2 h, remove MeCN by evaporation under reduced pressure, acidify with 5% HCl, purify by reverse phase HPLC using a 10 mM HCl/MeCN gradient (no further details).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS 80Ts (Tosoh)
Mobile phase: Gradient. A was 10 mM trifluoroacetic acid in water. B was 10 mM trifluoroacetic acid in MeCN:water 90:10. A:B from 100:0 to 85:15 over 1 min, to 75:25 over 9 min, to 65:35 over 10 min, to 0:100 over 10 min, maintain at 0:100 for 2 min, return to initial conditions over 1 min.
Injection volume: 100
Detector: F ex 485 em 540

CHROMATOGRAM

Retention time: 8
Limit of detection: 100 fmole

OTHER SUBSTANCES

Simultaneous: acetylcarnitine, butyrylcarnitine, decanoylcarnitine, heptanoylcarnitine, hexanoylcarnitine, isobutyrylcarnitine, isovalerylcarnitine, lauroylcarnitine, myristoylcarnitine, nonanoylcarnitine, octanoylcarnitine, palmitoylcarnitine, propionylcarnitine, stearoylcarnitine, valerylcarnitine, valproylcarnitine

KEY WORDS
derivatization; SPE

REFERENCE
Matsumoto,K.; Ichitani,Y.; Ogasawara,N.; Yuki,H.; Imai,K. Precolumn fluorescence derivatization of carnitine and acylcarnitines with 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole prior to high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *678*, 241–247.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount containing 25 mg carnitine chloride, add 40 mL water, shake for 10 min, make up to 50 mL, centrifuge at 3000 rpm for 5 min, dilute supernatant five times. 10 μL Diluted supernatant + 10 μL 20% aqueous sodium dodecyl sulfate + 10 μL 100 μg/mL N,N-dimethylglycine in water + 370 μL isopropanol + 100 μL 10 mg/mL 9-anthryldiazomethane in ethyl acetate, heat at 50° for 30 min, cool, inject a 55 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 10 μm LiChrosorb Si 100
Mobile phase: MeOH:5% aqueous sodium dodecyl sulfate:phosphoric acid 99:1:0.1
Flow rate: 1
Injection volume: 55
Detector: F ex 365 em 412 or UV 250

CHROMATOGRAM
Retention time: 10
Internal standard: N,N-dimethylglycine (5)
Limit of detection: 1 pg

OTHER SUBSTANCES
Noninterfering: antacids, ethyl aminobenzoate, thiamine, riboflavin, pyridoxine, ascorbic acid, niacinamide, pantothenic acid, dicycloverine, papaverine, methylbenactyzium

KEY WORDS
derivatization; tablets; normal phase

REFERENCE
Yoshida,T.; Aetake,A.; Yamaguchi,H.; Nimura,N.; Kinoshita,T. Determination of carnitine by high-performance liquid chromatography using 9-anthryldiazomethane, *J.Chromatogr.*, **1988**, *445*, 175–182.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out an appropriate amount, add 40 mL water, sonicate for 20 min, cool, make up to 50 mL, centrifuge at 1300 g for 10 min. remove a 2 mL aliquot of the supernatant and add it to a 150 × 10 column containing 2 g 100-200 mesh Amberlite CG-120 cation-exchange resin (Na^+ form), wash with 25 mL water, elute with 20 mL 2% ammonia solution, adjust the volume of the eluate to 25 mL with water. Remove a 500 μL aliquot and add it to 500 μL 50 μg/mL triamterene in MeCN:DMSO 99:1, evaporate to dryness under reduced pressure at 50°, reconstitute with 1 mL 200 μg/mL pyrene-1-carbonyl cyanide (Wako Chemicals, Richmond VA) in DMSO, heat at 80° for 30 min, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSKgel SP-2SW (Tosoh)
Mobile phase: MeCN:buffer 25:75 (Buffer was 10 mM $(NH_4)_2HPO_4$ adjusted to pH 7.5 with phosphoric acid.)
Column temperature: 40

Flow rate: 1
Injection volume: 1
Detector: F ex 355 em 420

CHROMATOGRAM
Retention time: 5
Internal standard: triamterene (8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: caffeine, cinnamon bark extract, coptis rhizome extract, EtOH, gentian extract, ginseng extract, glucuronolactone, glycyrrhizia extract, inositol, niacinamide, pantothenol, pyridoxine, riboflavin, sucrose, thiamine

KEY WORDS
derivatization; tablets; SPE

REFERENCE
Kamata,K.; Takahashi,M.; Terasima,K.; Nishijima,M. Liquid chromatographic determination of carnitine by precolumn derivatization with pyrene-1-carbonyl cyanide, *J.Chromatogr.A*, **1994**, *667*, 113–118.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out syrup, injections, or finely ground tablets containing 50 mg carnitine, add 40 mL water, sonicate for 20 min, cool, make up to 50 mL with water, centrifuge at 2000 rpm for 10 min. Remove a 1 mL aliquot and add it to 1 mL 100 μg/mL quinuclidine in MeCN, evaporate to dryness under reduced pressure at 50°, add 1 mL 2 mg/mL 9-anthroylnitrile (Wako) in DMSO, heat at 80° for 1.5 h, cool to room temperature, make up to 5 mL with DMSO, add a 100 μL aliquot to a 1 mL Bond Elut silica gel SPE cartridge, wash with 10 mL MeCN:MeOH 90:10, elute with 20 mL water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Ultron ES-OVM ovomucoid-conjugated (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 17:83 (Buffer was 20 mM KH_2PO_4 adjusted to pH 4.5 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7 (D), 10 (L)
Limit of detection: 50 μg/mL

KEY WORDS
derivatization; chiral; SPE; tablets; syrup; injections

REFERENCE
Takahashi,M.; Terashima,K.; Nishijima,M.; Kamata,K. Separation of carnitine enantiomers as the 9-anthroylnitrile derivatives and high-performance liquid chromatographic analysis on an ovomucoid-conjugated column, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1579–1584.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil NH2
Mobile phase: MeCN:20 mM pH 3 phosphate buffer 75:25
Flow rate: 1
Detector: UV 205

CHROMATOGRAM
Retention time: 8.65
Limit of quantitation: 185 μM

OTHER SUBSTANCES
Simultaneous: propionyl L-carnitine, crotonylbetaine, propionic acid

REFERENCE
Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E. Application of high-performance liquid chromatography to the analysis of propionyl-L-carnitine by a stereospecific enzyme assay, *J.Chromatogr.*, **1988**, *459*, 313–317.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL 200 μg/mL carnitine in 50 mM aqueous tetrabutylammonium phosphate + 200 μL 4.5 mM (+)-1-(9-fluorenyl)ethyl chloroformate in acetone, heat at 80° in a sealed vial for 25 min, cool, dilute with 4 mL MeCN:buffer 25:75, inject a 5 μL aliquot. (Buffer was 5 mM tetrabutylammonium hydroxide and 50 mM KH_2PO_4 adjusted to pH 7.0 with 1 M KOH.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:buffer 25:75. B was MeCN:5 mM KH_2PO_4 75:25. A:B 100:0 for 20 min, to 0:100 over 2 min, maintain at 0:100 for 13 min, to 100:0 over 1 min, maintain at 100:0 for 4 min. (Buffer was 5 mM tetrabutylammonium hydroxide and 50 mM KH_2PO_4 adjusted to pH 7.0 with 1 M KOH.)
Column temperature: 30
Flow rate: 0.75 for 20 min, then 1
Injection volume: 5
Detector: UV 260 or F ex 260 em 315

CHROMATOGRAM
Retention time: 12.5 (D), 14.5 (L)

KEY WORDS
derivatization

REFERENCE
De Witt,P.; Deias,R.; Muck,S.; Galletti,B.; Meloni,D.; Celletti,P.; Marzo,A. High-performance liquid chromatography and capillary electrophoresis of L- and D-carnitine by precolumn diastereomeric derivatization, *J.Chromatogr.B*, **1994**, *657*, 67–73.

SAMPLE
Matrix: solutions
Sample preparation: 500 μL 5 mg/mL carnitine in water + 100 mL acetone, remove a 1 mL aliquot and add it to 250 μL 1 mg/mL 9-anrthryldiazomethane in acetone, let stand at 50° for 20 min, evaporate the solvent under a stream of nitrogen, add 2 mL 0.1% perchloric acid to the residue, wash this solution twice with 6 mL diethyl ether. Remove 1 mL of the aqueous phase and add it to 1 mL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD-R (tris(3,5-dimethylphenylcarbamate)

Mobile phase: MeCN:500 mM sodium perchlorate solution 40:60
Flow rate: 0.8
Injection volume: 20
Detector: UV 254 or F ex 365 em 412

CHROMATOGRAM
Retention time: 16 (D), 18 (L)

KEY WORDS
derivatization; also for carnitine esters

REFERENCE
Hirota,T.; Minato,K.; Ishii,K.; Nishimura,N.; Sato,T. High-performance liquid chromatographic determination of the enantiomers of carnitine and acetylcarnitine on a chiral stationary phase, *J.Chromatogr.A*, **1994**, *673*, 37–43.

SAMPLE
Matrix: solutions
Sample preparation: 30 μL Carnitine in water + 30 μL 50 mM pH 10.4 carbonate buffer + 80 μL 15 mM (+)-[1-(9-fluorenyl)ethyl]chloroformate in acetone, heat at 45° for 1 h, add 90 μL 50 mM pH 4.2 acetate buffer, inject an aliquot.

HPLC VARIABLES
Column: 240 × 4.6 5 μm RP18
Mobile phase: MeCN:buffer 28.5:71.5 (Buffer was 6.8 mL triethylamine in 1 L water, adjust pH to 2.6 with 85% phosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: F ex 260 em 310

CHROMATOGRAM
Retention time: 15 (D), 17 (L)
Limit of detection: 0.5% of other enantiomer

KEY WORDS
chiral; derivatization

REFERENCE
Vogt,C.; Georgi,A.; Werner,G. Enantiomeric separation of D/L-carnitine using HPLC and CZE after derivatization, *Chromatographia*, **1995**, *40*, 287–295.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 250 μL 200 μM IS + 125 μL 1 M KOH, let stand for 10 min, add to the column, elute with 2.5 mL 500 mM ammonium hydroxide. Evaporate the eluate to dryness, reconstitute the residue in 100 μL 3 mM N,N-diisopropylethylamine in MeCN, vortex for 2 min, add 100 μL 7.5 mM 4'-bromophenacyl triflate in MeCN, vortex for 2 min, inject a 20 μL aliquot. (The column was 35 × 5 Dowex 50-X8 (NH_4^+ form) above 35 × 5 Dowex 1-X8 (OH^- form) in a Pasteur pipette. Convert Dowex 50-X8 (200-400 mesh, H^+ form) to the NH_4^+ form and 35 × 5 Dowex 1-X8 (200-400 mesh, Cl^- form) to the OH^- form according to instructions from Bio-Rad Labs. In particular conversion of Dowex 1-X8 must continue until tests for Cl^- in the column effluent are negative.)

HPLC VARIABLES
Guard column: 50 × 4 Co:Pell ODS
Column: 100 × 5 10 μm Radial-Pak C18

Mobile phase: MeCN:buffer 58:22 (The buffer was 3.56 g sodium dodecyl sulfate, 2.21 g KH_2PO_4, and 4.75 mL 3-(dimethylamino)-1,2-propanediol in 2.2 L water, adjust pH to 6.5 with concentrated phosphoric acid.)
Flow rate: 3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: 4-(N,N-dimethyl-N-(n-propyl)ammonio)-3-hydroxybutanoate (11.5)

OTHER SUBSTANCES
Extracted: butyrobetaine

KEY WORDS
SPE; derivatization

REFERENCE
Minkler,P.E.; Ingalls,S.T.; Hoppel,C.L. Determination of total carnitine in human urine by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 385–393.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg silica SPE cartridge (Baker) with 5 mL MeOH. 500 μL Urine + 100 nmoles IS in water, evaporate to dryness under reduced pressure, reconstitute with 500 μL MeOH, centrifuge at 10000 g for 3 min, add the supernatant to the SPE cartridge, wash with 2 mL MeOH, elute with 3 mL MeOH:water:acetic acid 45:50:5. Collect the final 2.5 mL of eluate and evaporate it to dryness under reduced pressure, reconstitute with 500 μL MeOH, centrifuge. Evaporate the supernatant to dryness under a stream of nitrogen in a clean tube at 40°, add 300 μL 6.8 mM N,N-diisopropylethylamine in MeCN, vortex for 10 s, sonicate for 15 min, add 200 μL 25 mM 4'-bromophenacyl bromide in MeCN, vortex for 10 s, heat at 37° for 30 min, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Hypersil BDS C8
Column: 200 × 4.6 5 μm Hypersil BDS C8
Mobile phase: Gradient. A was MeCN:water 70:30. B was MeCN:100 mM pH 5.0 triethylamine phosphate buffer 95:5. A:B 97:3 for 9 min, to 90:10 over 3 min, to 50:50 over 8 min, to 10:90 over 6 min, maintain at 10:90 for 3 min, return to initial conditions over 10 min, re-equilibrate for 5 min
Flow rate: 1.2 for 12 min, then 1.4
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 14
Internal standard: undecanoyl-L-carnitine (24)

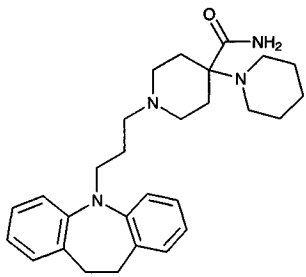
OTHER SUBSTANCES
Extracted: acetylcarnitine, butyrylcarnitine, decanoylcarnitine, hexanoylcarnitine, isovalerylcarnitine, nonanoylcarnitine, octanoylcarnitine, palmitoylcarnitine, propionylcarnitine

KEY WORDS
derivatization; SPE

REFERENCE

Poorthuis,B.J.H.M.; Jille-Vlckov,T.; Onkenhout,W. Determination of acylcarnitines in urine of patients with inborn errors of metabolism using high-performance liquid chromatography after derivatization with 4'-bromophenacylbromide, *Clin.Chim.Acta*, **1993**, *216*, 53–61.

Carpipramine



Molecular formula: $C_{28}H_{38}N_4O$
Molecular weight: 446.64
CAS Registry No.: 5942-95-0, 7075-03-8 (dihydrochloride monohydrate)
Merck Index: 1911
Lednicer No.: 2 416

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 251

CHROMATOGRAM

Retention time: 12.58
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine;

aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.18

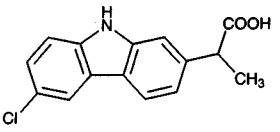
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Carprofen



Molecular formula: $C_{15}H_{12}ClNO_2$
Molecular weight: 273.72
CAS Registry No.: 53716-49-7
Merck Index: 1912
Lednicer No.: 2 169

SAMPLE

Matrix: bile, blood, urine

Sample preparation: Bile. Centrifuge sample, dilute 10-fold with 1% phosphoric acid, inject a 20 μL aliquot. Plasma. Vortex plasma, centrifuge, apply a 100-500 μL aliquot to a Varian C18 SPE cartridge. Wash with 500 gmL 1% phosphoric acid, wash with 500 μL MeOH: 1% phosphoric acid 20:80, elute with MeOH (?). Urine. Vortex urine, centrifuge, add a 500 μL aliquot to a Varian C18 SPE cartridge. Wash with 500 gmL 1% phosphoric acid, wash with 500 μL MeOH: 1% phosphoric acid 40:60, elute with MeOH (?).

HPLC VARIABLES

Column: 250 × 4.6 5 μm RP18 Kromasil (Interchim, France)

Mobile phase: Gradient. A. MeCN. B. 20 mM pH 5 NA_2HPO_4, 2 H_2O containing 10 mM citric acid. A:B 27:73 for 6 min, to 40:60 over 10 min.

Detector: F ex 310 em 375

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Extracted: carprofen glucuronides

KEY WORDS

plasma; dog; pharmacokinetics; SPE

REFERENCE

Priymenko,N.; Garnier,F.; Ferre,J.-P.; Delatour,P.; Toutain,P.-L. Enantioselectivity of the enterohepatic recycling of carprofen in the dog, *Drug Metab.Dispos.*, **1998**, *26*, 170–176.

SAMPLE

Matrix: blood

Sample preparation: Vortex plasma, centrifuge, apply a 100-500 μL aliquot to a Varian C18 SPE cartridge. Wash with 1 mL MeOH:water 20:80, elute with 1 mL MeOH. Evaporate the eluate to dryness, add 50 μL toluene, evaporate to dryness under nitrogen, reconstitute in 100 μL 50 mM triethylamine in dry MeCN, let stand for 1 min, add 50 μL 60 mM ethyl chloroformate in dry MeCN, mix, let stand for 1 min, add 50 μL 1 M L-leucinamide HCl in MeOH containing 1 M triethylamine, mix, let stand for 3 min, add 200 μL 250 mM HCl, extract with 4 mL ethyl acetate (J. Chromatogr. 1988, 433, 331), reconstitute with MeOH, pass through the same SPE cartridge, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm RP18 Kromasil (Interchim, France)

Mobile phase: Gradient. A. MeCN. B. 20 mM pH 5 NA_2HPO_4, 2 H_2O containing 10 mM citric acid. A:B from 40:60 to 60:40 over 12 min.

Detector: F ex 310 em 375 LQ 150 ng/mL

CHROMATOGRAM

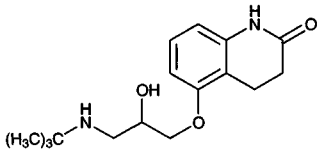
Retention time: 10 (R), 10.7 (S)

KEY WORDS
plasma; dog; chiral; pharmacokinetics; derivatization; SPE

REFERENCE

Priymenko,N.; Garnier,F.; Ferre,J.-P.; Delatour,P.; Toutain,P.-L. Enantioselectivity of the enterohepatic recycling of carprofen in the dog, *Drug Metab.Dispos.*, **1998**, *26*, 170–176.

Carteolol



Molecular formula: $C_{16}H_{24}N_2O_3$
Molecular weight: 292.38
CAS Registry No.: 51781-06-7, 51781-21-6 (HCl)
Merck Index: 1917
Lednicer No.: 3 183

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 251

CHROMATOGRAM

Retention time: 3.60

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Deproteinize plasma with perchloric acid, extract with dichloromethane, make extract alkaline with NaOH, extract with chloroform. Urine. Acidify urine with HCl, extract with chloroform, make extract alkaline with NaOH, extract with chloroform.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM $(NH_4)H_2PO_4$ and 20 mM $(NH_4)_2HPO_4$.)

Detector: UV 254

CHROMATOGRAM

Internal standard: 1-methylcarteolol

Limit of quantitation: 200 ng/mL (urine), 5 ng/mL (plasma)

KEY WORDS

plasma

REFERENCE

Odomi,M.; Akiyama,H.; Matsuura,K.; Shimizu,T. [The determination of carteolol in human plasma and urine by high performance liquid chromatography], *Yakugaku.Zasshi.*, **1985**, *105*, 459–463.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM
Retention time: 5.935

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL 20% sodium bicarbonate containing 500 μg/mL IS and 20 mg/mL sodium bisulfite, mix. Extract the mixture with 5 mL ethyl acetate. Remove the organic layer and dry it under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase. Inject a 20-30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80Ts (Tosoh Co., Japan)
Mobile phase: MeCN:water:acetic acid 10.5:89.5:1
Flow rate: 0.8
Injection volume: 20-30
Detector: UV 254

CHROMATOGRAM
Retention time: 17.7
Internal standard: p-aminobenzoic acid (11.7)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Umehara,K.; Kudo,S.; Odomi,M. Involvement of CYP2D1 in the metabolism of carteolol by male rat liver microsomes, *Xenobiotica*, **1997**, *27*, 1121–1129.

SAMPLE
Matrix: perfusate
Sample preparation: 50 μL Perfusate + 50 μL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 μL 20 μg/mL pindolol in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 25:75

Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Internal standard: pindolol

KEY WORDS
rabbit

REFERENCE
Sasaki,H.; Igarashi,Y.; Nagano,T.; Nishida,K.; Nakamura,J. Different effects of absorption promoters on corneal and conjunctival penetration of ophthalmic β-blockers, *Pharm.Res.*, **1995**, *12*, 1146–1150.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 internal surface reversed-phase silica (Pinkerton) (Regis Chemical)
Mobile phase: Isopropanol:100 mM pH 6.8 KH_2PO_4 10:90
Flow rate: 1
Injection volume: 10
Detector: UV 232-274 (wavelength of maximum absorption used)

CHROMATOGRAM
Retention time: 32.6

OTHER SUBSTANCES
Simultaneous: atenolol, metoprolol, acebutolol, oxprenolol, pindolol, alprenolol

REFERENCE
Ohshima,T.; Takagi,K.; Miyamoto,K.-I. High performance liquid chromatographic retention time of β-blockers as an index of pharmacological activity, *J.Liq.Chromatogr.*, **1993**, *16*, 3933–3939.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL 20 μg/mL pindolol in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 25:75
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Internal standard: pindolol

KEY WORDS
buffer; earle's balanced salt solution

REFERENCE
Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 60:40:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.48, 0.95 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.71

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g

powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/ 12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation

under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 35:65:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 23.4, 28.3 (enantiomers)
Limit of detection: 0.00635-0.008 fmole

OTHER SUBSTANCES

Also analyzed: atenolol, timolol

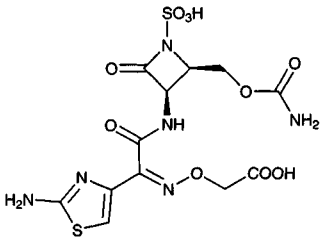
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Carumonam



Molecular formula: $C_{12}H_{14}N_6O_{10}S_2$
Molecular weight: 466.41
CAS Registry No.: 87638-04-8, 86832-68-0 (sodium salt)
Merck Index: 1922
Lednicer No.: 4 193

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 250 μL 100 mM pH 3.0 citrate buffer, vortex for 10 s, add 1 mL MeCN, vortex twice for 15 s, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex for 15 s, centrifuge at 1000 g for 10 min. Remove 200 μL of the aqueous phase and add it to 50 μL mobile phase, remove residual dichloromethane by evaporating under reduced pressure in a rotary evaporator (5 min at 200 Torr), inject a 40 μL aliquot into column A and column B in series, after 3.5 min remove column A from the circuit and backflush it with mobile phase to remove late-eluting peaks. Urine. 50 μL Urine + 200 μL 100 mM pH 5 phosphate buffer + 1 mL mobile phase, vortex, inject a 20 μL aliquot into column A and column B in series, after 3.5 min remove column A from the circuit and backflush it with mobile phase to remove late-eluting peaks.

HPLC VARIABLES

Column: A 30 × 4 5 μm Hypersil MOS; B 125 × 4 5 μm Hypersil ODS
Mobile phase: MeCN containing 4.5 g/L trihexylamine:water:50% sulfuric acid 30:70:0.1, adjusted to an apparent pH of 4.5 with 1 M NaOH
Flow rate: 1
Injection volume: 20-40
Detector: UV 293

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 25 μg/mL (urine), 500 ng/mL (plasma)

KEY WORDS

plasma; column-switching

REFERENCE

Egger,H.-J.; Fischer,G. Determination of the monocyclic β-lactam antibiotic carumonam in plasma and urine by ion-pair and ion-suppression reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 357–372.

SAMPLE

Matrix: cell suspensions
Sample preparation: Filter (0.45 μm).

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere IP ion pair
Mobile phase: MeOH:100 mM sodium perchlorate adjusted to pH 2.5 with concentrated sulfuric acid 35:65
Flow rate: 1
Injection volume: 20
Detector: UV 295

CHROMATOGRAM

Retention time: 5.0

OTHER SUBSTANCES
Extracted: cefpirome (UV 254)
Interfering: ceftriaxone (UV 254), cefotaxime (UV 254)

REFERENCE
Bellido,F.; Pechère,J.-C.; Hancock,R.E.W. Novel method for measurement of outer membrane permeability to new β-lactams in intact *Enterobacter cloacae* cells, *Antimicrob.Agents Chemother.*, **1991**, *35*, 68–72.

SAMPLE
Matrix: dialysate, ultrafiltrate, urine
Sample preparation: No details given.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:5 mM pH 3.0 tetrabutylammonium hydrogen sulfate 12:88
Flow rate: 0.8
Detector: UV 313

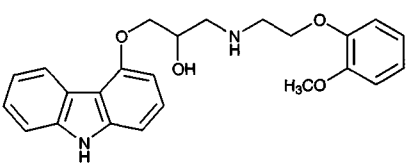
CHROMATOGRAM
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; rat; monkey; rabbit; dog

REFERENCE
Kita,Y.; Fugono,T.; Imada,A. Comparative pharmacokinetics of carumonam and aztreonam in mice, rats, rabbits, dogs, and cynomolgus monkeys, *Antimicrob.Agents Chemother.*, **1986**, *29*, 127–134.

Carvedilol



Molecular formula: $C_{24}H_{26}N_2O_4$
Molecular weight: 406.48
CAS Registry No.: 72956-09-3
Merck Index: 1924
Lednicer No.: 5 163

SAMPLE

Matrix: blood
Sample preparation: Condition a PrepSep RC18 SPE cartridge (Fisher) with 10 mL elution solvent and 2 mL MeCN:water 35:65. 1 mL Plasma + 1 mL 8 M guanidine hydrochloride, mix, add to SPE cartridge, wash with 2 mL MeCN:water 35:65, elute with two 200 μL portions of elution solvent. Add 50 μL 100 mM triethylamine in MeCN and 10 μL 20 mg/mL 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate in MeCN to the eluate, vortex briefly, allow to stand at room temperature for 30 min, add 150 μL 0.5% phosphoric acid, inject a 50-100 μL aliquot. (Elution solvent was MeCN:water:1 M triethylamine in water adjusted to pH 2.5 with phosphoric acid 80:17:3.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:water:1 M triethylamine in water adjusted to pH 2.5 with phosphoric acid 29:29:41.5:0.5
Flow rate: 1.6
Injection volume: 50-100
Detector: F ex 285 em 360

CHROMATOGRAM

Retention time: 9.5 (S-(-)), 11.5 (R-(+))
Internal standard: N,N-bis-carvedilol (4)
Limit of detection: 0.6 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; chiral; SPE; derivatization

REFERENCE

Eisenberg,E.J.; Patterson,W.R.; Kahn,G.C. High-performance liquid chromatographic method for the simultaneous determination of the enantiomers of carvedilol and its O-desmethyl metabolite in human plasma after chiral derivatization, *J.Chromatogr.*, **1989**, *493*, 105–115.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 ng IS in MeOH + 1 mL 100 mM pH 8 Britton-Robinson buffer + 5 mL ether, shake for 10 min at ca. 60 strokes/min, centrifuge at 1500 g for 10 min. Remove the organic layer and add it to 300 μL 50 mM sulfuric acid, shake, centrifuge. Remove the aqueous layer and add it to 1 mL 100 mM pH 8 Britton-Robinson buffer, add 5 mL ether, shake for 10 min, centrifuge. Remove the organic layer and dry it over 300 mg $MgSO_4.7H_2O$ (sic) by vortexing for a few s and standing for 10 min. Evaporate the ether solution to dryness under a stream of nitrogen. Take up the residue in 200 μL MeCN:triethylamine 99.6:0.4 and add 5 μL 25.2 mM 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, let stand at room temperature for 10 min, evaporate under

a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm ODS (Brownlee)
Column: 150 × 4.6 5 μm ODS 80TM (Tosoh)
Mobile phase: MeOH:EtOH:2 mM pH 7.0 $(NH_4)_2HPO_4$ 60:4:36
Column temperature: 50
Flow rate: 2.5
Injection volume: 50
Detector: F ex 285 em 355

CHROMATOGRAM
Retention time: 5.4 (R-(+)), 6.3 (S-(-))
Internal standard: 1-(o-methoxyphenyl)-4-[3-(naphthyloxy)-2-hydroxypropyl]piperazine (7.8)
Limit of detection: 1.55 ng/mL

KEY WORDS
plasma; human; monkey; derivatization; pharmacokinetics

REFERENCE
Fujimaki,M.; Murakoshi,Y.; Hakusui,H. Assay and disposition of carvedilol enantiomers in humans and monkeys: evidence of stereoselective presystemic metabolism, *J.Pharm.Sci.*, **1990**, *79*, 568–572.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL pH 9.8 buffer + 500 mg NaCl + 5 mL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 30 min, centrifuge at 4000 g for 10 min. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL toluene, evaporate to dryness, add 100 μL 400 μg/mL S-naproxen chloride in anhydrous dichloromethane, heat at 50° for 1 h, evaporate to dryness, reconstitute with 120 μL mobile phase, inject a 100 μL aliquot. Urine. 1 mL Urine (or plasma) + 1 mL pH 5 sodium citrate buffer + 10 μL (5.500 Fishman units) β-glucuronidase, heat at 37° for 4 h, add 200 μL 100 mM NaOH, add 1 mL buffer, add 500 mg NaCl, add 5 mL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 30 min, centrifuge at 4000 g for 10 min. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL toluene, evaporate to dryness, add 100 μL 400 μg/mL S-naproxen chloride in anhydrous dichloromethane, heat at 50° for 1 h, evaporate to dryness, reconstitute with 120 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by mixing 42 mL 100 mM sodium carbonate with 58 mL 100 mM sodium bicarbonate, pH 9.8. Synthesis of S-naproxen chloride is as follows. Protect all compounds from light. Dissolve 500 mg naproxen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 1 h, evaporate to dryness under reduced pressure, dry over KOH under vacuum overnight to obtain S-naproxen chloride (mp 96°) (Arch. Pharm. (Weinheim) 1988, 321, 847).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: n-Hexane:dichloromethane:EtOH 112:36:1.7
Flow rate: 1.5
Injection volume: 100
Detector: F ex 285 em 355

CHROMATOGRAM
Retention time: k' 13.3 (S-(-)), k' 15.7 (R-(+))
Limit of detection: 1 ng/mL

KEY WORDS
derivatization; plasma; chiral; normal phase; pharmacokinetics

REFERENCE
Spahn,H.; Henke,W.; Langguth,P.; Schloos,J.; Mutschler,E. Measurement of carvedilol enantiomers in human plasma and urine using S-naproxen chloride for chiral derivatization, *Arch.Pharm.(Weinheim)*, **1990**, *323*, 465–469.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
. . .**Detector:** UV 254

CHROMATOGRAM
Retention time: k' 6.11, k' 7.35 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 250 × 4.6 Brownlee RP-300 C8

Mobile phase: Gradient. A was 100 mM pH 5.0 ammonium acetate. B was MeCN:water 80:20. A:B from 90:10 to 55:45 over 70 min, to 0:100 over 5 min, maintain at 0:100 for 5 min.
Flow rate: 1
Detector: UV 285

CHROMATOGRAM
Retention time: 60

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; rat; liver

REFERENCE
Schaefer,W.H. Formation of a carbamoyl glucuronide conjugate of carvedilol in vitro using dog and rat liver microsomes, *Drug Metab.Dispos.*, **1992**, *20*, 130–133.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Centrifuge mixture at 1000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Capcell Pak C18 AG120 (Shiseido)
Mobile phase: MeOH:100 mM pH 9.0 phosphate buffer containing 4% triethylamine 55:45
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 330 em 380

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 28 nmole/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Fujimaki,M. Oxidation of R(+) and S(-)-carvedilol by rat liver microsomes. Evidence for stereoselective oxidation and characterization of cytochrome P450 isozymes involved, *Drug Metab.Dispos.*, **1994**, *22*, 700–708.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 50 μL DMF, make up to 500 μL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OF
Mobile phase: n-Hexane:isopropanol 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 50

Detector: UV 254

CHROMATOGRAM
Retention time: 9.5 (R-(+)), 18.3 (S-(-))

KEY WORDS
chiral

REFERENCE
Fujimaki,M.; Shintani,S.; Hakusui,H. Stereoselective metabolism of carvedilol in the rat. Use of enantiomerically radiolabeled pseudoracemates, *Drug Metab.Dispos.*, **1991**, *19*, 749–753.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 3.33 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 13.96

OTHER SUBSTANCES
Also analyzed: bisoprolol, labetalol, metipranolol, oxprenolol, talinolol, toliprolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a

short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES

Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 100
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM

Retention time: k' 7.1 (S-(-)), 8.0 (R-(+))

OTHER SUBSTANCES

Simultaneous: flecainide (no enantiomeric separation), propafenone

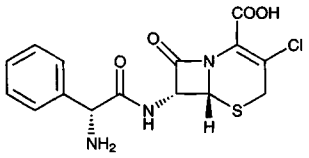
KEY WORDS

derivatization; chiral; F not much more sensitive than UV; α = 1.13

REFERENCE

Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

Cefaclor



Molecular formula: $C_{15}H_{14}ClN_3O_4S$
Molecular weight: 367.81
CAS Registry No.: 53994-73-3, 70356-03-5 (monohydrate)
Merck Index: 1962
Lednicer No.: 3 209

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 2

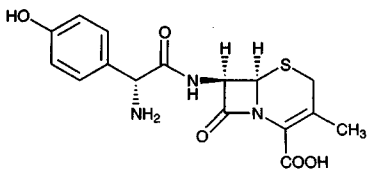
OTHER SUBSTANCES

Simultaneous: 7-ACA, 7-ADCA, cefaloridine, cefazolin, cefoperazone, cefotaxime, ceftazidime, cephalosporin C

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

Cefadroxil



Molecular formula: $C_{16}H_{17}N_3O_5S$
Molecular weight: 363.39
CAS Registry No.: 66592-87-8
Merck Index: 1963
Lednicer No.: 2 440

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL water + 200 μL 1 M pH 5 acetate buffer, vortex briefly. Filter (Centrifree micropartition unit) 1 mL mixture while centrifuging at 2000 g for 10 min. Inject a 25 μL aliquot of the ultrafiltrate. Urine. 1 mL urine + 1 mL 10 mM pH 3.5 acetate buffer + 1 mL 10% tetrabutylammonium hydroxide (adjusted to pH 2 with sulfuric acid) + 7.5 mL water saturated mixture of n-amyl alcohol:dichloromethane 2:1, mix on a flat-bed shaker at 100 strokes/min for 15 min, centrifuge at 2500 g for 5 min. Remove organic phase and add 7.5 mL water saturated mixture of n-amyl alcohol:dichloromethane 2:1. Briefly vortex a 250 μL aliquot of the upper aqueous phase with 4.75 mL water. Inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleogil C18
Mobile phase: MeCN:10 mM phosphoric acid adjusted to pH 3 with NaOH 6:94 (plasma) or MeCN:10 mM pH 3.0 phosphoric acid 5:95 (urine)
Flow rate: 1
Injection volume: 25
Detector: UV 260

CHROMATOGRAM

Retention time: 5.6 (plasma) or 7.0 (urine)
Limit of quantitation: 200 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Barbhaiya,R.H. A pharmacokinetic comparison of cefadroxil and cephalexin after administration of 250, 500 and 1000 mg solution doses, *Biopharm.Drug Dispos.*, **1996**, *17*, 319–330.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 150 μL Plasma + 150 μL MeCN, vortex, rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min. Transfer supernatant to another tube and add 7 volumes dichloromethane, equilibrate for 10 min; rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min, inject an aliquot of the upper aqueous layer (J.Chromatogr. 1987, 413, 109). Urine. Dilute with water, inject an aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 1.6 Spherisorb S5-ODS2 C18
Mobile phase: MeOH:100 mM pH 3 acetate buffer 13:87
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Limit of detection: 300 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Gimeno,M.J.; Martínez,M.; Granero,L.; Torres-Molina,F.; Peris,J.-E. Influence of probenecid on the renal excretion mechanisms of cefadroxil, *Drug Metab.Dispos.*, **1996**, *24*, 270–272.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.188

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: React a sample with 300 mM HCl at 24° for 2 h or with 10 mM NaOH at ambient temperature for 1.5 h or with 1 M NaOH for 0.5 h or by heating the solid at 140° for 6 h or by heating a solution at 40° for 8 h.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeCN. B was 2 mM ammonium acetate. A:B 2:98 for 30 min, from 2:98 to 20:80 in 10 min, from 20:80 to 50:50 in 10 min
Flow rate: 1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 18.31

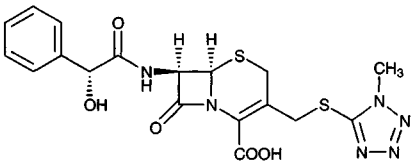
OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Rourick,R.A.; Volk,K.J.; Klohr,S.E.; Spears,T.; Kerns,E.H.; Lee,M.S. Predictive strategy for the rapid structure elucidation of drug degradants, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1743–1752.

Cefamandole



Molecular formula: $C_{18}H_{18}N_6O_5S_2$
Molecular weight: 462.51
CAS Registry No.: 34444-01-4, 42540-40-9 (nafate), 30034-03-8 (sodium)
Merck Index: 1964
Lednicer No.: 2 441

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 7.8 (mobile phase A), 10.3 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 8.2
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 15 μg/mL

OTHER SUBSTANCES
Extracted: cefazolin, cefotaxime, cefoxitin, cephapirin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 19.2:0.8:80, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: cephalothin (8)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Cool blood in ice. 1 mL Blood + 100 μL pH 5.0 sodium acetate buffer + 100 μL 0.05% tri-o-tolyl phosphate in MeOH, centrifuge, remove plasma, add 10 μL glacial acetic acid. 200 μL Plasma + 200 μL 20 μg/mL cephalothin in 50 mM phosphoric acid, mix, keep at 4° before injection, inject a 100 μL aliquot onto column A with mobile phase A, elute column A with mobile phase A for 10 min, backflush the contents of column A onto column B with mobile phase B for 4 min, remove column A from the circuit, continue to elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 15 min. Urine. 1.5 mL Urine + 0.5 mL pH 3.0 2 M sodium acetate buffer. Dilute with 20 μg/mL cephalothin in 50 mM phosphoric acid so as to have an appropriate concentration of cefamandole, mix, keep at 4° before injection, inject a 100 μL aliquot onto column A with mobile phase A,

elute column A with mobile phase A for 10 min, backflush the contents of column A onto column B with mobile phase B for 4 min, remove column A from the circuit, continue to elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 15 min.

HPLC VARIABLES

Column: A 40 × 2 37-50 μm Corasil RP C18; B 40 × 4.6 37-53 μm Co:Pell ODS + 250 × 4 10 μm LiChrosorb RP-8
Mobile phase: A 50 mM phosphoric acid; B MeOH:5 mM tetrabutylammonium bromide 45:55
Flow rate: A 1; B 1
Injection volume: 100
Detector: UV 270

CHROMATOGRAM

Retention time: 9 (cefamandole), 16 (cefamandole nafate)
Internal standard: cephalothin (14)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: cefotiam, cephalexin, cephaloridine, caffeine, cefotaxime, cefuroxime, cefazolin, cefoperazone, cefoxitin
Noninterfering: phenobarbital

KEY WORDS

plasma; column-switching

REFERENCE

Lee,H.S.; Zee,O.P.; Kwon,K.I. Simultaneous determination of cefamandole and cefamandole nafate in human plasma and urine by high-performance liquid chromatography with column switching, *J.Chromatogr.*, **1990**, *528*, 425–433.

SAMPLE

Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 100 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 20 (cefamandole), 48 (cefamandole nafate)
Limit of quantitation: 6400 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cephalothin

REFERENCE

Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 20:80
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 270

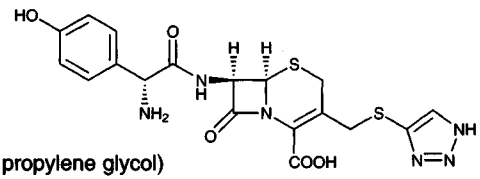
OTHER SUBSTANCES

Also analyzed: cephapirin

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefatrizine



Molecular formula: $C_{18}H_{18}N_6O_5S_2$
Molecular weight: 462.51
CAS Registry No.: 51627-14-6, 64217-62-5 (compd with propylene glycol)
Merck Index: 1965
Lednicer No.: 3 211

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.808

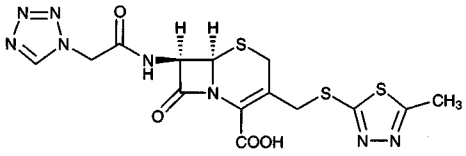
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cefazolin



Molecular formula: $C_{14}H_{14}N_8O_4S_3$
Molecular weight: 454.51
CAS Registry No.: 25953-19-9, 27164-46-1 (sodium salt)
Merck Index: 1967
Lednicer No.: 3 442

SAMPLE

Matrix: blood
Sample preparation: Condition an 800 µL 500 mg Sep-Pak Vac 3cc C18 SPE cartridge twice with 800 µL MeOH and with 800 µL water. 200 µL Serum + 200 µL water, vortex. Add to the SPE cartridge. Wash twice with 800 µL water. Elute twice with 400 µL MeCN: 50 mM KH_2PO_4 70:30 and twice with 400 µL MeCN:water 50:50. Evaporate the eluate under a stream of nitrogen at 50° for 10 min. Cool, centrifuge at 1450-1475 g for 4 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: KGCQ-324C (YMC, Wilmington, NC)
Column: 250 × 4.6 5 µm YMC pack ODS-AQ (YMC, Wilmington, NC)
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 6 phosphate buffer 5:4:91. B was MeCN:MeOH:50 mM pH 6 phosphate buffer 8:8:84. A:B 100:0 for 2 min, to 0:100 over 9 min, maintain at 0:100 for 14 min, to 100:0 over 5 min, maintain at 100:0 for 5 min (Prepare buffer as follows. Dissolve 11.94 g KH_2PO_4 and 2.14 g K_2HPO_4 in 2 L water.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 27
Internal standard: cefazolin

OTHER SUBSTANCES

Extracted: vancomycin
Simultaneous: acetaminophen, salicylates, theophylline

KEY WORDS

serum; SPE; cefazolin is IS

REFERENCE

Backes,D.W.; Aboleneen,H.I.; Simpson,J.A. Quantitation of vancomycin and its crystalline degradation product (CDP-1) in human serum by high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1281–1287.

SAMPLE

Matrix: blood
Sample preparation: Mix 100 µL plasma with 40 µL 5% trichloroacetic acid in MeOH. Vortex for 10 s, add 50 µL 30 µg/mL IS. Vortex for 10 s, centrifuge at 5000 rpm for 10 min, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18
Mobile phase: MeCN:50 mM KH_2PO_4 7:93
Flow rate: 3
Detector: UV 273

CHROMATOGRAM
Retention time: 14
Internal standard: cefoxitin (11)
Limit of detection: 500 ng/mL
Limit of quantitation: 2 μg/mL

KEY WORDS
plasma; pharmacokinetics; rat

REFERENCE
Allababidi,S.; Shah,J.C. Efficacy and pharmacokinetics of site-specific cefazolin delivery using biodegradable implants in the prevention of post-operative wound infections, *Pharm.Res.*, **1998**, *15*, 325–333.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM
Retention time: 5.0 (mobile phase A), 6.0 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 *g* for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine

Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 3.6
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 3 μg/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefotaxime, cefoxitin, cephapirin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 13.44:0.56:86, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: cefoxitin (4)
Limit of detection: 200 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL 8.5% phosphoric acid. Condition an NH2 SPE cartridge with 1 mL hexane. 500 μL Plasma + 25 μL 8.5% phosphoric acid + 250 μL 1 mg/mL coumarin-3-carboxylic acid in water, add to the C18 SPE cartridge, wash with 500 μL water, wash with 1 mL 8.5% phosphoric acid, wash with 5% MeOH:8.5% phosphoric acid 20:1, elute with 1 mL MeOH: 8.5% phosphoric acid 60:40 into the NH2 SPE cartridge. Wash the NH2 SPE cartridge

with 1 mL hexane, wash with 1 mL MeCN, elute with 1 mL water:10% ammonium sulfate 95:5, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES

Column: 250 × 4.6 C18
Mobile phase: Water:2 mM tetramethylammonium hydroxide in MeOH:acetic acid 60:40:0.5
Flow rate: 0.8
Injection volume: 20
Detector: UV 262

CHROMATOGRAM

Retention time: 10
Internal standard: coumarin-3-carboxylic acid (13)

OTHER SUBSTANCES

Extracted: ceftizoxime, cefaclor, cephalexin

KEY WORDS

plasma; SPE

REFERENCE

Moore,C.M.; Sato,K.; Hattori,H.; Katsumata,Y. Improved HPLC method for the determination of cephalosporins in human plasma and a new solid-phase extraction procedure for cefazolin and ceftizoxime [letter], *Clin.Chim.Acta*, **1990**, *190*, 121–123.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 8.423

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 40 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 11
Limit of quantitation: 1600 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefoxitin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE

Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE

Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 5.09 KH_2PO_4 10:90
Flow rate: 1
Injection volume: 75
Detector: UV 254

CHROMATOGRAM

Retention time: 11
Internal standard: Vancomycin
Limit of detection: 100-1000 ng/mL

REFERENCE

Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE

Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak phenyl
Mobile phase: MeCN:MeOH:0.5% phosphoric acid with 0.7% triethylamine 10:20:70
Flow rate: 1.5
Injection volume: 20
Detector: UV 322

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Noninterfering: meperidine

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Lee,D.K.T.; Wong,C.-Y.; Wang,D.-P. Stability of cefazolin sodium and meperidine hydrochloride, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1608–1610.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeOH:water 30:70 containing 10 mM ammonium acetate
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: cephalothin

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE
Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak C18
Mobile phase: MeOH:5 mM pH 7.5 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Simultaneous: degradation products, cefoxitin

KEY WORDS
injections; water; stability-indicating

REFERENCE
Stiles,M.L.; Tu,Y.H.; Allen,L.V.,Jr. Stability of cefazolin sodium, cefoxitin sodium, ceftazidime, and penicillin G sodium in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1989**, *46*, 1408–1412.

SAMPLE
Matrix: formulations
Sample preparation: Add theophylline (2 mg/mL), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:acetic acid:water 10:1:89, adjusted to pH 4 with 5 M NaOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 293 (?)

CHROMATOGRAM
Retention time: 8.5
Internal standard: theophylline (4.1)

OTHER SUBSTANCES
Simultaneous: ceftazidime

KEY WORDS
injections; stability-indicating; 5% dextrose

REFERENCE
Bosso,J.A.; Prince,R.A.; Fox,J.L. Compatibility of ondansetron hydrochloride with fluconazole, ceftazidime, aztreonam, and cefazolin sodium under simulated Y-site conditions, *Am.J.Hosp.Pharm.*, **1994**, *51*, 389–391.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with 5% dextrose (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: Nova Pak C18
Mobile phase: MeOH:100 mM $(NH_4)_2HPO_4$ 20:80, pH 7.80
Flow rate: 1
Injection volume: 20
Detector: UV 322

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: famotidine

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE

Wang,D.-P.; Chang,L.-C.; Wong,C.-Y.; Lee,D.K.T. Stability of cefazolin sodium-famotidine admixture, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2205–2209.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in a mobile phase to concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 54

OTHER SUBSTANCES

Also analyzed: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefoperazone, cefotaxime, ceftazidime, cephalosporin C

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:90 mM perchloric acid 13.5:86.5. B was MeCN:300 mM perchloric acid 45:55. A:B from 100:0 to 0:100 over 7 min, maintain at 0:100.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: 7-aminocephalosproranic acid, cefadroxil, cefotaxime, cephalexin, cephaloridine, cephalosporin C, cephalothin, cephapirin, D-hydroxyphenylglycine

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: solutions
Sample preparation: Inject 100 μL onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 0.3 5 μm ODS C18 (Nomura); B 150 × 0.3 5 μm ODS C18 (Nomura)
Mobile phase: A 10 mM ammonium acetate adjusted to pH 5 with acetic acid; B MeOH: water:acetic acid 40:60:0.5
Flow rate: A 0.1; B 0.004
Injection volume: 100
Detector: UV 262

CHROMATOGRAM
Retention time: 8.50
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: cefaclor, cephaloridine, ceftizoxime

KEY WORDS
microbore; column-switching

REFERENCE
Moore,C.M.; Sato,K.; Katsumata,Y. High-performance liquid chromatographic determination of cephalosporin antibiotics using 0.3 mm I.D. columns, *J.Chromatogr.*, **1991**, *539*, 215–220.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 270

OTHER SUBSTANCES
Also analyzed: cefpiramide, cefmenoxime, cefbuperazone, cefoxitin, cefotiam, cephaloridine

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 2.5-5 μg/mL solution, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 3.65 μm Zorbax Rx-SIL (similar to Zorbax SB-C8 (Mac-Mod Analytical))
Mobile phase: MeCN:0.1% trifluoroacetic acid 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 272

CHROMATOGRAM
Retention time: k' 2.9

REFERENCE

Kirkland,K.M.; McCombs,D.A.; Kirkland,J.J. Rapid, high-resolution high-performance liquid chromatographic analysis of antibiotics, *J.Chromatogr.A*, **1994**, *660*, 327–337.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 Spheri 5 ODS-224

Mobile phase: 100 mM sodium dodecyl sulfate, pH 3.00

Flow rate: 1

Injection volume: 10

Detector: UV 260

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: cephalothin, cephaloridine, cephalexin, cephradine, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE

Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 50-200 μL aliquot of a solution in pH 7.4 Tyrode's buffer.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 60 RP-select B

Mobile phase: MeCN:10 mM pH 4 sodium acetate 12:88

Flow rate: 0.6

Injection volume: 50-200

Detector: UV 270

OTHER SUBSTANCES

Also analyzed: theophylline

KEY WORDS

buffer

REFERENCE

Saitoh,H.; Aungst,B.J. Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine, *Pharm.Res.*, **1995**, *12*, 1304–1310.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 100 mg Sep-Pak SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize tissue with 4 (liver, lung) or 29 (spleen) volumes of water (Thomas tissue grinder series 3431-D70). 1 mL Homogenate + 20 μL 500 μg/mL cephalexin + 50 μL 8.5% phosphoric acid, vortex for 30 s, centrifuge at 2000 g for 5 min, add to the SPE cartridge, wash with 3 mL water, elute with 2 mL MeOH:water 60:40, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES

Guard column: Nova-Pak C18 guard column
Column: 250 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:20 mM NaH_2PO_4 23:77, pH 5.0
Flow rate: 1
Injection volume: 100
Detector: UV 270

CHROMATOGRAM

Retention time: 9.5
Internal standard: cephalexin (11.5)
Limit of quantitation: 500 ng/g (spleen), 100 ng/g (liver, lung)

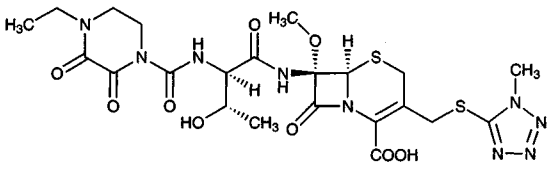
KEY WORDS

rat; liver; spleen; lung; SPE

REFERENCE

Liang,D.; Chow,D.; White,C. High-performance liquid chromatographic assay of cefazolin in rat tissues, *J.Chromatogr.B*, **1994**, *656*, 460–465.

Cefbuperazone



Molecular formula: $C_{22}H_{29}N_9O_9S_2$
Molecular weight: 627.66
CAS Registry No.: 76610-84-9
Merck Index: 1968
Lednicer No.: 4 189

SAMPLE

Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 270

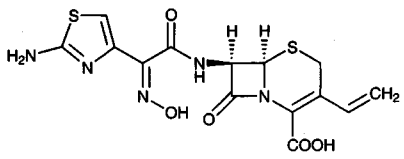
OTHER SUBSTANCES

Also analyzed: cefpiramide, cefazolin, cefmenoxime, cefoxitin, cefotiam, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefdinir



Molecular formula: $C_{14}H_{13}N_5O_5S_2$
Molecular weight: 395.42
CAS Registry No.: 91832-40-5
Merck Index: 1971

SAMPLE

Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, mix with 3.5 volumes of dichloromethane, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: MeCN:water:perchloric acid:triethylamine 8:90.6:0.7:0.7
Flow rate: 1
Injection volume: 20
Detector: UV 284

CHROMATOGRAM

Retention time: 11
Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES

Also analyzed: captopril, quinapril

KEY WORDS

plasma; pharmacokinetics; rat

REFERENCE

Jacolot,A.; Tod,M.; Petitjean,O. Pharmacokinetic interaction between cefdinir and two angiotensin-converting enzyme inhibitors in rats, *Antimicrob.Agents Chemother.*, **1996**, *40*, 979–982.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve 200 mg cefdinir in 5 mL 2% sodium bicarbonate in water. Dilute 500 μL solution to 200 mL (100 μg/mL) with each 100 mM pH 3-8 phosphate buffer. For pH 9 studies dissolve 20 mg cefdinir in 200 mL 100 mM carbonate buffer. Inject an aliquot.

HPLC VARIABLES

Column: 75 × 4.6 TSK-gel ODS-80 TM (TOSOH, Japan)
Mobile phase: MeOH:dioxane:33 mM citric acid adjusted to pH 2.0 with 10% phosphoric acid 4:1:36 (Caution ! Dioxane is a carcinogen!)
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Retention time: 6.5

OTHER SUBSTANCES

Simultaneous: degradation products

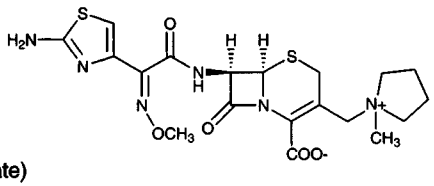
KEY WORDS

details for degradation of cefdinir in paper

REFERENCE

Okamoto,Y.; Kiriyama,K.; Namiki,Y.; Matsushita,J.; Fujioka,M.; Yasuda,T. Degradation kinetics and isomerization of cefdinir, a new oral cephalosporin, in aqueous solution. 1, *J.Pharm.Sci.*, **1996**, *85*, 976–983.

Cefepime



Molecular formula: $C_{19}H_{24}N_6O_5S_2$

Molecular weight: 480.57

CAS Registry No.: 88040-23-7, 123171-59-5 (HCl monohydrate)

Merck Index: 1973

SAMPLE

Matrix: blister fluid, blood

Sample preparation: Serum. 0.5 mL Serum + 2.5 mL MeCN, vortex, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex, inject a 10 μL aliquot of the aqueous layer. Blister fluid. Inject directly.

HPLC VARIABLES

Column: μBondapak C18

Mobile phase: MeCN:100 mM phosphate buffer 7:93

Flow rate: 2

Injection volume: 10

Detector: UV 229

CHROMATOGRAM

Retention time: 5

KEY WORDS

serum; pharmacokinetics

REFERENCE

Kalman,D.; Barriere,S.L.; Johnson,B.L.,Jr. Pharmacokinetic disposition and bactericidal activities of cefepime, ceftazidime, and cefoperazone in serum and blister fluid, *Antimicrob.Agents Chemother.*, **1992**, *36*, 453–457.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Serum + 500 μL MeCN, vortex, gently shake by rotation at 20 rpm for 10 min. and centrifuge at 1000 g for 10 min. Remove the supernatant, add 3.2 mL dichloromethane, shake by rotation at 20 rpm for 10 min. and centrifuge at 1000 g for 10 min. Inject a 5 μL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere XL-ODS

Mobile phase: MeCN:20 mM ammonium acetate adjusted to pH 4 with glacial acetic acid 7:93

Flow rate: 1

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 1.8-2.2

Limit of detection: 100 ng/mL

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Noninterfering: amikacin, amoxicillin, ampicillin, calcium folinate, cephalothin cefixime, cefotaxime, ceftazidime, ciprofloxacin, clavulanic acid, cloxacillin, erythromycin, fosfomycin, fusidic acid, gentamycin, hydroxyitraconazole, imipenem, itraconazole, kanamycin, latamoxef, lincomycin, mezlocillin, neomycin, netilmycin, ofloxacin, pefloxacin, penicillin G, piperacillin, pristinamycin, rifampicin, roxithromycin, sulbactam, sulfamethoxazole, tazobactam, teicoplanin, tetracycline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum; pharmacokinetics

REFERENCE

Elkhaïli,H.; Linger,L.; Monteil,H.; Jehl,F. High-performance liquid chromatographic assay for cefepime in serum, *J.Chromatogr.B*, **1997**, *690*, 181–188.

SAMPLE

Matrix: blood

Sample preparation: 500 µL Serum + 900 µL MeCN:water:trichloroacetic acid 50:40:1.5 (v/v/w) + 1.5 mL dichloromethane, vortex for 10 s, centrifuge at 1200 g for 20 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 5 µm C18
Mobile phase: MeCN:3.5% glycine adjusted to pH 9.5 with NaOH 5:95
Flow rate: 1
Injection volume: 50
Detector: UV 280 (from ref. 6)

CHROMATOGRAM

Limit of detection: 40 ng/mL

KEY WORDS

serum; dog

REFERENCE

Stampley,A.R.; Brown,M.P.; Gronwall,R.R.; Castro,L.; Stone,H.W. Serum concentrations of cefepime (BMY-28142), a broad-spectrum cephalosporin, in dogs, *Cornell.Vet.*, **1992**, *82*, 69–77.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 µL Plasma + 100 µL 100 µg/mL cefadroxil + 300 µL 5% trichloroacetic acid + 500 µL MeCN + 1.5 mL dichloromethane, vortex for 10 s, centrifuge at 500-600 g at 5° for 10 min, inject a 25 µL aliquot of the aqueous supernatant. Urine. Dilute urine three-fold with 200 mM pH 4.5 sodium acetate buffer, add 100 µL 1500 µg/mL ceftazidime, vortex for 30 s, inject a 10 µL aliquot.

HPLC VARIABLES

Guard column: 23 × 4 37-50 µm Corasil C18
Column: 150 × 4 Nova-Pak (plasma) or 100 × 9.4 Partisil 5 ODS-3RAC C18 (urine)
Mobile phase: MeCN:5 mM 1-octanesulfonic acid 12:88 (plasma) or MeOH:10 mM sodium dodecyl sulfate adjusted to pH 3.0 with glacial acetic acid:5% trichloroacetic acid:850 mM phosphoric acid:THF 49.7:40.4:3.9:0.7:5.3 (urine)
Flow rate: 1 (plasma), 2.8 (urine)
Injection volume: 10-25
Detector: UV 280

CHROMATOGRAM

Retention time: 7 (plasma), 7.5 (urine)

Internal standard: cefadroxil (10) (plasma), ceftazidime (10) (urine)
Limit of quantitation: 2000 ng/mL (urine), 100 ng/mL (plasma)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Barbhaiya,R.H.; Forgue,S.T.; Shyu,W.C.; Papp,E.A.; Pittman,K.A. High-pressure liquid chromatographic analysis of BMY-28142 in plasma and urine, *Antimicrob.Agents Chemother.*, **1987**, *31*, 55–59.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 750 μL Plasma + 75 μL 200 mM pH 6.0 4-morpholineethanesulfonic acid buffer, mix, add 1.1 mL MeCN, centrifuge. Remove the supernatant and evaporate the MeCN under a stream of nitrogen, inject a 100 μL aliquot of the residue. Urine. Inject 100-200 μL urine directly.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 5:95 containing 13 mM sodium pentanesulfonate, 11 mM sodium heptanesulfonate, and 10 mM sodium acetate, pH 3.0
Flow rate: 1
Injection volume: 100-200
Detector: Radioactivity

CHROMATOGRAM
Retention time: 45

KEY WORDS
plasma; rat; dog; radiolabeled

REFERENCE
Forgue,S.T.; Kari,P.; Barbhaiya,R. *N*-oxidation of *N*-methylpyrrolidine released *in vivo* from cefepime, *Drug Metab.Dispos.*, **1987**, *15*, 808–815.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Precipitate protein in serum with MeCN and trichloroacetic acid, add dichloromethane, mix, centrifuge. Dilute aqueous supernatant with pH 4 sodium acetate buffer, inject an aliquot. Urine. Dilute urine with pH 4 sodium acetate buffer, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP18
Column: 125 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeCN:water:concentrated sulfuric acid 800:1197.5:2.5 containing 20 mM sodium dodecyl sulfate, adjust pH to 2.3 with concentrated sulfuric acid
Detector: UV 260

CHROMATOGRAM
Limit of detection: 1800 ng/mL, 270 ng/mL

KEY WORDS
serum

REFERENCE

Bächer,K.; Schaeffer,M.; Lode,H.; Nord,C.E.; Borner,K.; Koeppe,P. Multiple dose pharmacokinetics, safety, and effects on faecal microflora, of cefepime in healthy volunteers, *J.Antimicrob.Chemother.*, **1992**, *30*, 365–375.

SAMPLE

Matrix: cecal contents

Sample preparation: Dilute cecal contents in 2 mL phosphate buffered saline, centrifuge at 1500 g for 10 min. 500 μL Sample + 300 μL 5% trichloroacetic acid + 500 μL MeCN + 1.5 mL dichloromethane, vortex for 10 s, centrifuge at 500-600 g at 5° for 10 min, inject a 20 μL aliquot of the upper aqueous phase.

HPLC VARIABLES

Column: 100 × 3 5 μm Hypersil ODS

Mobile phase: MeCN: 5 mM pH 5.5 acetate buffer 0.7:99.3

Flow rate: 1

Injection volume: 20

Detector: UV 280

KEY WORDS

mouse; pharmacokinetics

REFERENCE

van Ogtrop,M.L.; Guiot,H.F.L.; Mattie,H.; van Furth,R. Modulation of the intestinal flora of mice by parenteral treatment with broad-spectrum cephalosporins, *Antimicrob.Agents Chemother.*, **1991**, *35*, 976–982.

SAMPLE

Matrix: cell suspensions

Sample preparation: Filter (0.45 μm).

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere IP ion pair

Mobile phase: MeOH:100 mM sodium perchlorate adjusted to pH 2.5 with concentrated sulfuric acid 30:70

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Also analyzed: cefpirome, carumonam (UV 295), ceftriaxone, cefotaxime

REFERENCE

Bellido,F.; Pechère,J.-C.; Hancock,R.E.W. Novel method for measurement of outer membrane permeability to new β-lactams in intact *Enterobacter cloacae* cells, *Antimicrob.Agents Chemother.*, **1991**, *35*, 68–72.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize muscle with three volumes phosphate buffered saline (Polytron, level 3) for 2 min, centrifuge at 1300 g for 10 min. 125 μL Supernatant + 100 μL 40 μg/mL cefadroxil in water + 800 μL MeCN, vortex for 30 s, centrifuge at 1600 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 125 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 4 μm Novapak C18
Mobile phase: MeCN:5 mm sodium heptanesulfonic acid 9:91, adjust pH to 3.33 with glacial acetic acid
Flow rate: 2
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 4
Internal standard: cefadroxil (6.7)
Limit of detection: 800 ng/g, 200 ng/mL

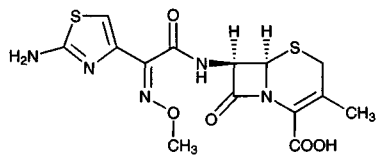
KEY WORDS

mouse; muscle

REFERENCE

Darouiche,R.; Musher,D.; Hamill,R.; Ou,C.; Rognerud,C. Cephalosporin penetration into soft tissue of paralyzed limbs, *Antimicrob.Agents Chemother.*, **1989**, *33*, 1326–1328.

Cefetamet



Molecular formula: $C_{14}H_{15}N_5O_5S_2$
Molecular weight: 397.44
CAS Registry No.: 65052-63-3
Merck Index: 1974
Lednicer No.: 4 184

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 500 mM perchloric acid, vortex for 5 s, let stand for 15 min, centrifuge at 1500 g for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 Nucleosil 5 C18
Mobile phase: MeCN:100 mM pH 6.5 phosphate buffer 40:60
Flow rate: 1
Injection volume: 50 (cefetamet pivoxyl)
Detector: UV 265

CHROMATOGRAM

Retention time: 5.6
Limit of detection: 200-400 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS

plasma; human; dog; rat

REFERENCE

Wyss,R.; Bucheli,F. Determination of cefetamet and its orally active ester, cefetamet pivoxyl, in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 81–92.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 500 mM perchloric acid, vortex for 5 s, let stand for 15 min, centrifuge at 1500 g for 5 min, inject a 30 μL aliquot of the supernatant. Urine. Dilute 500 μL urine to 25 mL with water, vortex for 5 s, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Spherisorb ODS 1
Mobile phase: MeCN:4 mM perchloric acid 17:83 (plasma) or 15:85 (urine)
Flow rate: 1
Injection volume: 30
Detector: UV 265

CHROMATOGRAM

Retention time: 8 (plasma), 11 (urine)
Limit of quantitation: 20 μg/mL (urine), 200 ng/mL (plasma)

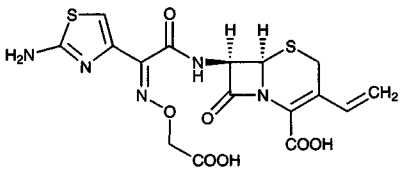
KEY WORDS

plasma; human; dog; rat

REFERENCE

Wyss,R.; Bucheli,F. Determination of cefetamet and its orally active ester, cefetamet pivoxyl, in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 81–92.

Cefixime



Molecular formula: $C_{16}H_{15}N_5O_7S_2$
Molecular weight: 453.46
CAS Registry No.: 79350-37-1
Merck Index: 1975
Lednicer No.: 4 184,185

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 287.8

CHROMATOGRAM

Retention time: 4.823

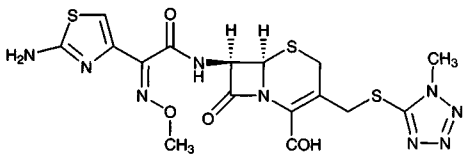
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cefmenoxime



Molecular formula: $C_{16}H_{17}N_9O_5S_3$
Molecular weight: 511.57
CAS Registry No.: 65085-01-0, 75738-58-8 (HCl)
Merck Index: 1976
Lednicer No.: 4 187

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 12:88 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 7.6
Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 7.8 μg/mL IS in 4% sodium dodecyl sulfate, centrifuge using an Amicon Model 25 filter (MW cutoff 25000) at 450 g for 20 min, inject a 90 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:200 mM pH 5.30 acetate buffer 13:87
Flow rate: 2
Injection volume: 90
Detector: UV 254

CHROMATOGRAM

Retention time: 6

Internal standard: p-nitrobenzoic acid (3) or p-anisic acid (7)
Limit of detection: 50 ng/mL

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Granneman,G.R.; Sennello,L.T. A very precise high-performance liquid chromatographic procedure for the determination of cefmenoxime, a new cephalosporin antibiotic, in plasma, *J.Chromatogr.*, **1982**, *229*, 149–157.

SAMPLE
Matrix: blood, CSF
Sample preparation: Serum. 1 mL Serum + 1 mL MeCN, vortex, centrifuge at 5000 rpm for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex, centrifuge. Remove the aqueous supernatant and inject an aliquot. CSF. Inject CSF directly.

HPLC VARIABLES
Column: endcapped 5 μm Lichrospher RP 18
Mobile phase: MeCN:2% acetic acid 25:75
Flow rate: 1
Injection volume: 4.61
Detector: UV 254

CHROMATOGRAM
Retention time: 20

KEY WORDS
serum

REFERENCE
Condomines,M.; Mallet,M.N.; Albanese,J.; Gouin,F.; De Micco,P. A rapid high-performance liquid chromatography method for determining β-lactam antibiotics in biological fluids and tissues, *Chemioterapia*, **1987**, *6*, 251–253.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 1 mL 10 (serum) or 100 (urine) μg/mL cefuroxime in MeOH, shake on a microthermomixer for 30 s, filter (urine samples only), centrifuge at 3000 rpm for 3 min, filter (0.5 μm) the supernatant, dilute with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 Nucleosil 5C18
Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:water:acetic acid 10:50:1
Flow rate: 0.7
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: cefuroxime (11)
Limit of detection: 2000 ng/mL (urine), 50 ng/mL (serum)

KEY WORDS
serum

REFERENCE
Itakura,K.; Mitani,M.; Aoki,I.; Usui,Y. High performance liquid chromatographic assay of cefsulodin, cefotiam and cefmenoxime in serum and urine, *Chem.Pharm.Bull.(Tokyo)*, **1982**, *30*, 622–627.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL 0.8 μg/mL p-anisic acid in MeCN, vortex for 10 sa, centrifuge at 700 g for 10 min. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen, vortex briefly, inject a 25-50 μL aliquot. Urine. Dilute, add p-anisic acid (200 μg/mL), inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:0.2% phosphoric acid 14:86
Flow rate: 2
Injection volume: 25-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Internal standard: p-anisic acid (14)
Limit of detection: 5000 ng/mL (urine), 200 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Noonan,I.A.; Gambertoglio,J.G.; Barriere,S.L.; Conte,J.E.,Jr.; Lin,E.T. High-performance liquid chromatographic determination of cefmenoxime (AB-50912) in human plasma and urine, *J.Chromatogr.*, **1983**, *273*, 458–463.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 100-fold. 500 μL Serum, plasma, or diluted urine + 100 μL 24 μg/mL p-anisic acid in water, vortex for 30 s, add 100 μL perchloric acid, vortex for 30 s, centrifuge at 2000 rpm for 15 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak CN
Mobile phase: pH 3.8 Buffer prepared from 109 mL 13.61 g/L sodium acetate trihydrate solution + 891 mL 5.75 mL/L acetic acid solution. (After each batch run MeOH through column at 3 mL/min for 10 min.)
Flow rate: 2.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 3.4
Internal standard: p-anisic acid (5.6)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, cimetidine, diazepam, digoxin, dopamine, furosemide, heparin, hydralazine, hydrochlorothiazide, hydrocortisone, hydromorphone, isosorbide dinitrate, methyldopa, nitroglycerin, quinidine, theophylline, tobramycin

KEY WORDS
serum; plasma; pharmacokinetics

REFERENCE

Reitberg,D.P.; Schentag,J.J. Liquid-chromatographic assay of cefmenoxime in serum and urine, *Clin.Chem.*, **1983**, *29*, 1415–1418.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 260

OTHER SUBSTANCES

Also analyzed: cefpiramide, cefazolin, cefbuperazone, cefoxitin, cefotiam, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE

Matrix: surface wipes

Sample preparation: Swab 100 × 100 mm surface with 1% pH 6 phosphate buffer (total volume 10 mL), remove excess liquid with a second swab, vortex swabs for 45 s, filter (0.45 μm polycarbonate), inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 15:100:1
Flow rate: 2
Injection volume: 100
Detector: UV 254

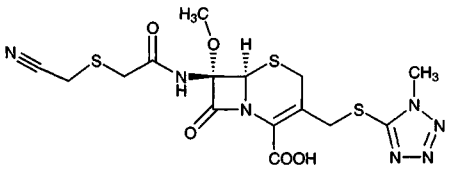
CHROMATOGRAM

Retention time: 7.1
Limit of quantitation: 100 ng/mL

REFERENCE

Gorski,R.J.; Plasz,A.C.; Elrod,L.J.; Yoder,J.; White,L.B. Determination of cefsulodin, cefmenoxime, and cefadroxil as residues on surfaces, *Pharm.Res.*, **1991**, *8*, 1525–1527.

Cefmetazole



Molecular formula: $C_{15}H_{17}N_7O_5S_3$
Molecular weight: 471.54
CAS Registry No.: 56796-20-4, 56796-39-5 (sodium salt)
Merck Index: 1977
Lednicer No.: 4 190

SAMPLE

Matrix: blood, urine
Sample preparation: Blood. Add 500 μL MeOH to 500 μL serum, vortex for 30 s, centrifuge at 10000 g for 15 min, inject a 50 μL aliquot of the supernatant. Urine. Add 4.5 mL 50 mM pH 6.0 potassium phosphate buffer to 500 μL urine, mix vigorously for 15 s, filter 1 mL of this solution (0.45 μm filter, Millex HA, Millipore), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Nucleosil-5 C18
Column: 150 × 4.6 5 μm Nucleosil-5 C18
Mobile phase: A MeCN:100 mM pH 6.0 potassium phosphate buffer 13:87; B MeCN:100 mM pH 6.0 potassium phosphate buffer 20:80 containing 0.1 mM hexadecyltrimethylammonium
Flow rate: 1
Injection volume: 50
Detector: UV 272

CHROMATOGRAM

Retention time: 7.4 (A), 7.7 (B)
Limit of quantitation: 200 ng/mL (serum), 2 μg/mL (urine)

KEY WORDS

serum

REFERENCE

García-Glez,J.C.; Méndez,R.; Martín-Villacorta,J. Quantitative determination of semisynthetic cephamycins in human serum and urine by ion-exchange, reversed-phase and ion-pair chromatography, *J.Chromatogr.A*, **1998**, *812*, 197–204.

SAMPLE

Matrix: blood, urine
Sample preparation: Mix, deproteinize, add barbital, vortex, centrifuge, inject an aliquot.

HPLC VARIABLES

Column: C18
Mobile phase: MeCN:20 mM pH 5.4 citrate buffer 12:88
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Internal standard: barbital
Limit of quantitation: 15000 ng/mL (urine), 2000 ng/mL (plasma)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Borin,M.T.; Peters,G.R.; Smith,T.C. Pharmacokinetics and dose proportionality of cefmetazole in healthy young and elderly volunteers, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1944–1948.

SAMPLE

Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: Nova Pak C18
Mobile phase: MeCN:0.1% acetic acid:10 mM pH 7.8 $(NH_4)H_2PO_4$ 10:23:74
Flow rate: 1
Injection volume: 20
Detector: UV 300

CHROMATOGRAM

Retention time: 8.1

OTHER SUBSTANCES

Simultaneous: famotidine
Noninterfering: degradation products

KEY WORDS

stability-indicating; injections; 5% dextrose

REFERENCE

Lee,D.K.T.; Wong,C.-Y.; Wang,D.-P.; Chang,L.-C.; Wu,K.-H. Stability of cefmetazole sodium and famotidine, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 432–442.

SAMPLE

Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 19.7 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 18:82 (Buffer was 20 mM phosphoric acid containing 10 mM sodium decanesulfonate.)
Flow rate: 1
Injection volume: 200
Detector: UV 260

REFERENCE

Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate + 10 mM tetrabutylammonium bromide + 1% acetic acid 25:75
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 270

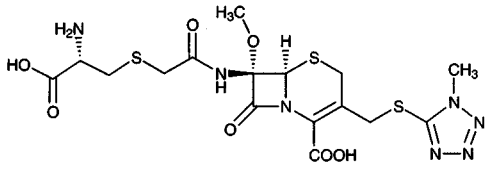
OTHER SUBSTANCES

Also analyzed: cefpiramide, cefoperazone, cefmenoxime, ceftezole

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefminox



Molecular formula: $C_{16}H_{21}N_7O_7S_3$
Molecular weight: 519.58
CAS Registry No.: 84305-41-9, 75481-73-1
Merck Index: 1978

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. Add 500 μL MeOH to 500 μL serum, vortex for 30 s, centrifuge at 10000 g for 15 min, inject a 50 μL aliquot of the supernatant. Urine. Add 4.5 mL 50 mM pH 6.0 potassium phosphate buffer to 500 μL urine, mix vigorously for 15 s, filter 1 mL of this solution (0.45 μm filter, Millex HA, Millipore), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Nucleosil-5 C18
Column: 150 × 4.6 5 μm Nucleosil-5 C18
Mobile phase: A MeCN:100 mM pH 6.0 potassium phosphate buffer 3:97; B MeCN:100 mM pH 6.0 potassium phosphate buffer 7:93 containing 0.1 mM hexadecyltrimethylammonium
Flow rate: 1
Injection volume: 50
Detector: UV 272

CHROMATOGRAM

Retention time: 7.8 (A), 6.2 (B)
Limit of quantitation: 200 ng/mL (serum), 2 μg/mL (urine)

KEY WORDS

serum

REFERENCE

García-Glez,J.C.; Méndez,R.; Martín-Villacorta,J. Quantitative determination of semisynthetic cephamycins in human serum and urine by ion-exchange, reversed-phase and ion-pair chromatography, *J.Chromatogr.A*, **1998**, *812*, 197–204.

SAMPLE

Matrix: solutions

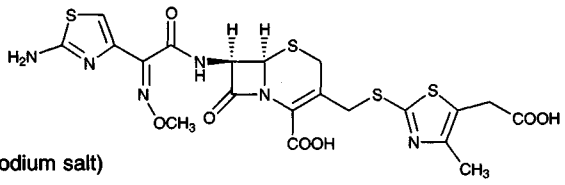
HPLC VARIABLES

Column: 100 × 4 5 μm ODS-Hypersil
Mobile phase: MeOH:water:o-phosphoric acid 20:80:1
Flow rate: 2
Detector: UV 270

REFERENCE

Soriano,F.; Edwards,R.; Greenwood,D. Comparative susceptibility of cefminox and cefoxitin to β-lactamases of *Bacteroides* spp., *J.Antimicrob.Chemother.*, **1991**, *28*, 55–60.

Cefodizime



Molecular formula: $C_{20}H_{20}N_6O_7S_4$
Molecular weight: 584.68
CAS Registry No.: 69739-16-8, 86329-79-5 (disodium salt)
Merck Index: 1979

SAMPLE

Matrix: bile, blood, feces, tissue, urine

Sample preparation: Plasma, serum. 500 µL Plasma or serum + 2 mL ice-cold 2.5 µg/mL 3,5-dinitrobenzoic acid in MeOH, vortex for 30 s, place in an ice-bath for 30 min, centrifuge at 2000 g in a refrigerated centrifuge for 15 min. Remove the supernatant and concentrate it under nitrogen to 500 µL, inject a 20 µL aliquot. Urine. 1 mL Centrifuged urine + 4 mL 25 µg/mL 3,5-dinitrobenzoic acid in 100 mM pH 7.0 phosphate buffer, mix well, filter (0.45 µm), inject a 20 µL aliquot. Bile. 500 µL Centrifuged bile + 1 mL 15 µg/mL 3,5-dinitrobenzoic acid in 100 mM pH 7.0 phosphate buffer, stir, filter (0.45 µm), inject a 20 µL aliquot. Feces. 1 g Homogenized feces + 4 mL ice-cold EtOH:1% pH 6.0 phosphate buffer 2:1, shake vigorously for 5 min, centrifuge in a refrigerated centrifuge at 2000 g for 10 min, filter (0.45 µm) the supernatant. 500 µL Supernatant + 500 µL 10 µg/mL 3,5-dinitrobenzoic acid in 100 mM pH 7.0 phosphate buffer, mix, inject a 20 µL aliquot. Tissue. Homogenize 0.1-1 g visceral tissue with 4 mL 100 mM pH 7.0 phosphate buffer, centrifuge at 5° at 2000 g for 15 min. 500 µL Supernatant + 2 mL ice-cold 5 µg/mL 3,5-dinitrobenzoic acid in MeOH, vortex vigorously, let stand at 5° for 30 min, centrifuge at 5° at 2000 g for 15 min. Remove the supernatant and concentrate it to 500 µL under nitrogen, inject a 20 µL aliquot. (Prepare 100 mM pH 7.0 phosphate buffer with 35.81 $Na_2HPO_4.12H_2O$ and 13.61 g KH_2PO_4 in 1 L water, adjust pH with NaOH or phosphoric acid if necessary. Prepare 1% pH 6.0 phosphate buffer with 6.0 g $Na_2HPO_4.12H_2O$ and 7.0 g KH_2PO_4 in 1 L water, adjust pH with NaOH or phosphoric acid if necessary.)

HPLC VARIABLES

Column: 100 × 8 4 µm Radial-Pak NOVA C18
Mobile phase: MeCN:water:acetic acid 20:80:2 containing 5 mM sodium 1-hexanesulfonate
Flow rate: 2
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 7.7
Internal standard: 3,5-dinitrobenzoic acid (10.2)
Limit of detection: 300 ng/mL (bile), 500 ng/mL (urine), 500 ng/g (feces, tissue), 100 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: piperacillin, cefmetazole, cefotetan, cefoperazone, cefotaxime

KEY WORDS

plasma; serum; human; rat; liver; spleen; lung; kidney; heart; brain; intestine; stomach; thymus

REFERENCE

Marunaka,T.; Matsushima,E.; Maniwa,M. Determination of cefodizime in biological materials by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 329–339.

SAMPLE

Matrix: blood

Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 5.7 (mobile phase A), 6.8 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: Dilute plasma with three volumes of mobile phase A, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 1 min elute the contents of column A on to column B (already equilibrated with mobile phase A) with mobile phase A, after 2.5 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B. Before the next injection equilibrate columns A and B with mobile phase A.

HPLC VARIABLES

Column: A 50 × 4 40 μm CN silica; B 200 × 4 5 μm HP ODS (Hewlett-Packard)
Mobile phase: A MeCN:5 mM sodium 1-heptanesulfonate:acetic acid 10:88:2; B MeCN:5 mM sodium 1-heptanesulfonate:acetic acid 27:71:2
Flow rate: 1
Injection volume: 100
Detector: UV 263

CHROMATOGRAM

Retention time: 7.6
Limit of detection: 100 ng/mL

KEY WORDS

column-switching; plasma; pharmacokinetics

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L.; de Martinis,M.; Ginaldi,L.; Quaglino,D. Determination of cefodizime in human plasma by high-performance liquid chromatography with column-switching, *J.Liq.Chromatogr.*, **1995**, *18*, 2895–2909.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 250 μL Plasma + uvitic acid + 500 μL MeOH, centrifuge, add to SAX-modified silica SPE cartridge, wash with water, elute with 500 μL 1 M NaCl, add 500 μL water to the eluate, inject a 500 μL aliquot. Urine. 100 μL Urine + uvitic acid + 900 μL 100 mM pH 5.0 acetate buffer, add to SAX-modified silica SPE cartridge, wash with water, elute with 500 μL 1 M NaCl, add 500 μL water to the eluate, inject a 500 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:100 mM pH 4.0 citrate buffer 14:86 (plasma) or 13:87 (urine)

Flow rate: 1.5

Injection volume: 500

Detector: UV 300

CHROMATOGRAM

Retention time: 13

Internal standard: uvitic acid (9.5)

Limit of quantitation: 1 μg/mL (urine), 20 ng/mL (plasma)

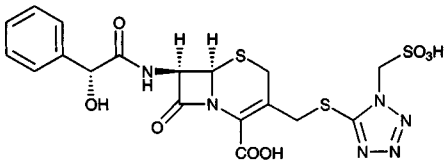
KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Lenfant,B.; Namour,F.; Logeais,C.; Coussediere,D.; Rivault,O.; Bryskier,A.; Surjus,A. Pharmacokinetics of cefodizime following single doses of 0.5, 1.0, 2.0, and 3.0 grams administered intravenously to healthy volunteers, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2037–2041.

Cefonicid



Molecular formula: $C_{18}H_{18}N_6O_8S_3$
Molecular weight: 542.57
CAS Registry No.: 61270-58-4, 61270-78-8 (disodium salt), 71420-79-6 (monosodium salt)
Merck Index: 1980
Lednicer No.: 3 213

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma, serum. 200 μL Plasma or serum + 400 μL 2.1 μg/mL cephalothin in MeCN:water 5:1, vortex for 10 s, centrifuge at 1500 g for 10 min. Remove the supernatant and evaporate it to 200 μL under nitrogen, vortex, inject a 5-20 μL aliquot. Urine. 200 μL Urine + 400 μL 8.3 μg/mL cephalothin in MeCN:water 5:1, vortex for 10 s, centrifuge at 1500 g for 10 min. Remove the supernatant and inject a 5-20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:water:phosphoric acid:tetrabutylammonium hydrogen sulfate 25:74.6:0.1:0.3
Flow rate: 2
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.5
Internal standard: cephalothin (11.5)
Limit of detection: 500 ng/mL

KEY WORDS

plasma; serum

REFERENCE

Phelps,R.; Zurlinden,E.; Conte,J.E.,Jr.; Lin,E. High-performance liquid chromatographic determination of cefonicid in human plasma, serum and urine, *J.Chromatogr.*, **1986**, *375*, 111–118.

SAMPLE

Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 100 μL (500 μL for urine) 10 μg/mL cefoperazone in 100 mM pH 5 ammonium acetate buffer, vortex for 15 s, centrifuge at 8700 g for 2 min, inject a 20-200 μL aliquot onto column A with mobile phase A, wash with mobile phase A for 2 min then backflush contents of column A onto column B with mobile phase B, monitor effluent from column B.

HPLC VARIABLES

Column: A 20 × 4 35-50 μm C18 Corasil; B 20 × 4 35-50 μm C18 Corasil + 100 × 8 10 μm Radial-Pak μBondapak C18
Mobile phase: A water:triethylamine 1000:4, adjusted to pH 3.0 with orthophosphoric acid; B MeCN:water:triethylamine 750:250:4, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: A 2; B 3.8
Injection volume: 20-200
Detector: UV 270

CHROMATOGRAM
Retention time: 2.33
Internal standard: cefoperazone (3.66)
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Simultaneous: ceftazidime, ceftriaxone, cefotaxime, cephaloridine, ceforanide, moxalactam, cephalothin
Noninterfering: cefotiam, cefadroxil
Interfering: cefazolin

KEY WORDS
plasma; column-switching

REFERENCE
Demotes-Mainard,F.; Vinçon,G.; Jarry,C.; Necciari,J.; Albin,H. Micromethod for the determination of cefpiramide in human plasma and urine by high-performance liquid chromatography using automated column switching, *J.Chromatogr.*, **1987**, *419*, 388–395.

SAMPLE
Matrix: solutions
Sample preparation: Add 100 μL solution to 1 mL 0.5 mg/mL cefoperazone in water, vortex for 15 s, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C8
Mobile phase: MeCN:20 mM sodium acetate 30:70 containing 1.7 g/L tetrabutylammonium hydrogen sulfate, pH adjusted to 6.0 with 1 M NaOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

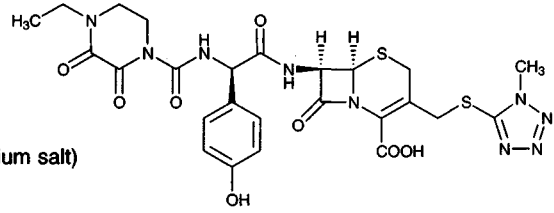
CHROMATOGRAM
Retention time: 8.5
Internal standard: cefoperazone (5.3)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
5% dextrose; saline

REFERENCE
Marble,D.A.; Bosso,J.A.; Townsend,R.J. Compatibility of clindamycin phosphate with aztreonam in polypropylene syringes and with cefoperazone sodium, cefonicid sodium, and cefuroxime sodium in partial-fill glass bottles, *Drug Intell.Clin.Pharm.*, **1988**, *22*, 54–57.

Cefoperazone



Molecular formula: $C_{25}H_{27}N_9O_8S_2$
Molecular weight: 645.68
CAS Registry No.: 62893-19-0, 62893-20-3 (sodium salt)
Merck Index: 1981
Lednicer No.: 4 185, 188-190

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 20:80 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Dilute bile and urine with water. 50 μL Plasma, diluted urine, or diluted bile + 50 μL 10% perchloric acid + 50 μL 3-butylxanthine in pH 7.4 phosphate buffer, mix, centrifuge at 15000 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeOH:30 mM pH 5.0 KH_2PO_4 20:80
Column temperature: 50
Flow rate: 1.5
Detector: UV 266

CHROMATOGRAM

Internal standard: 3-butylxanthine
Limit of detection: 100 ng/mL
Limit of quantitation: 200 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Haghgoo,S.; Hasegawa,T.; Nadai,M.; Wang,L.; Nabeshima,T.; Kato,N. Effect of a bacterial lipopolysaccharide on biliary excretion of a β-lactam antibiotic, cefoperazone, in rats, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2258–2261.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM
Retention time: 6.0 (mobile phase A), 7.8 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefodizime, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 18.24:0.76:81, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4

Internal standard: cephaloridine (3)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 150 μL MeOH, vortex for 30 s, incubate at room temperature for 5 min, centrifuge at 1500 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Develosil ODS-10
Column: 250 × 4.6 10 μm Develosil ODS-10
Mobile phase: MeOH:5 mM Na_2HPO_4 + 5 mM NaH_2PO_4 1:2
Flow rate: 1.2
Injection volume: 20
Detector: UV 265

KEY WORDS
plasma; human; rat

REFERENCE
Haginaka,J.; Wakai,J.; Yasuda,H.; Uno,T.; Nakagawa,T. High-performance liquid chromatographic assay of sulbactam using pre-column reaction with 1,2,4-triazole, *J.Chromatogr.*, **1985**, *341*, 115–122.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL ice-cold 100 μg/mL ceftriaxone in MeOH: 100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, hold at -20° for 10 min, centrifuge at 1500 g for 10 min, inject 15 μL of supernatant.

HPLC VARIABLES
Guard column: 10 μm C18 Guard-PAK
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM pH 7.5 phosphate buffer containing 10 mM hexadecyltrimethylammonium bromide 25:75
Flow rate: 1.3
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Internal standard: ceftriaxone
Limit of detection: 800 ng/mL

KEY WORDS
serum

REFERENCE
Deeter,R.G.; Weinstein,M.P.; Swanson,K.A.; Gross,J.S.; Bailey,L.C. Crossover assessment of serum bactericidal activity and pharmacokinetics of five broad-spectrum cephalosporins in the elderly, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1007–1013.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL MeCN, mix vigorously on a Whirlmixer for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μL of the supernatant and add it to 3 mL dichloromethane, centrifuge at 1200 g for 5 min, inject a 20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: 100 × 3 5 μm Hypersil ODS
Mobile phase: MeCN:5 mM pH 5.5 acetate buffer 0.7:97.3
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Also analyzed: ceftriaxone, ceftazidime, cefepime

KEY WORDS
plasma; mouse

REFERENCE
van Ogtrop,M.L.; Mattie,H.; Guiot,H.F.L.; van Strijen,E.; Hazekamp-van Dokkum,A.-M.; van Furth,R. Comparative study of the effects of four cephalosporins against Escherichia coli in vitro and in vivo, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1932–1937.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 1 g tissue with 10 mL pH 6.0 100 mM phosphate buffer (Polytron homogenizer), centrifuge at 3000 g for 10 min. 0.5 mL Serum or tissue homogenate supernatant + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex , centrifuge at 3000 g for 5 min, inject a 50 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 300 mm long μBondapak C18
Mobile phase: MeCN:100 mM sodium phosphate 16:84, adjust pH to 6.0
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Also analyzed: cefoxitin

KEY WORDS
serum

REFERENCE
Bawdon,R.E.; Hemsell,D.L.; Guss,S.P. Comparison of cefoperazone and cefoxitin concentrations in serum and pelvic tissue of abdominal hysterectomy patients, *Antimicrob.Agents Chemother.*, **1982**, *22*, 999–1003.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 1 mL Serum + 1 mL MeOH, vortex for 30 s, allow to stand at room temperature for 10 min, if necessary dilute with water to give a cefoperazone concentration of 1-100 μg/mL, centrifuge at 1022 g for 10 min, inject a 20 μL aliquot. Urine. Centrifuge at 1022 g for 10 min, if necessary dilute with water to give a cefoperazone concentration of 1-100 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: Gradient. A was 1.2 mM triethylamine and 42 mM acetic acid. B was MeCN: water 24:76 containing 1.2 mM triethylamine and 42 mM acetic acid. A:B from 75:25 to 60:40 using Waters Model 660 curve select-9 over 15 min then stay at 60:40.
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 23
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, ampicillin, methicillin, penicillin G
Noninterfering: gentamicin, kanamycin, tobramycin

KEY WORDS
serum

REFERENCE
Dokladalova,J.; Quercia,G.T.; Stankewich,J.P. High-performance liquid chromatographic determination of cefoperazone in human serum and urine, *J.Chromatogr.*, **1983**, *276*, 129–137.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 (?) μL Plasma or urine + 1500 (?) μL MeCN, vortex, centrifuge, inject a 20 μL aliquotof the supernatant.

HPLC VARIABLES
Column: Brownlee 10 μm RP-8
Mobile phase: MeCN:MeOH:20 mM pH 5 phosphate buffer 10:20:70
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Also analyzed: ceftriaxone, cefaronide

KEY WORDS
plasma; sheep; pharmacokinetics

REFERENCE
Guerrini,V.H.; Filippich,L.J.; Cao,G.R.; English,P.B.; Bourne,D.W.A. Pharmacokinetics of cefaronide, ceftriaxone and cefoperazone in sheep, *J.Vet.Pharmacol.Ther.*, **1985**, *8*, 120–127.

SAMPLE

Matrix: blood, urine

Sample preparation: 100 μL Plasma or urine + 100 μL (500 μL for urine) 100 mM pH 5 ammonium acetate buffer, vortex for 15 s, centrifuge at 8700 g for 2 min, inject a 20-200 μL aliquot onto column A with mobile phase A, wash with mobile phase A for 2 min then backflush contents of column A onto column B with mobile phase B, monitor effluent from column B.

HPLC VARIABLES

Column: A 20 × 4 35-50 μm C18 Corasil; B 20 × 4 35-50 μm C18 Corasil + 100 × 8 10 μm Radial-Pak μBondapak C18

Mobile phase: A water:triethylamine 1000:4, adjusted to pH 3.0 with orthophosphoric acid; B MeCN:water:triethylamine 750:250:4, adjusted to pH 3.0 with orthophosphoric acid

Flow rate: A 2; B 3.8

Injection volume: 20-200

Detector: UV 270

CHROMATOGRAM

Retention time: 3.66

Internal standard: cefoperazone

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: cefonicid, ceftazidime, ceftriaxone, cefotaxime, cephaloridine, ceforanide, moxalactam, cephalothin

Noninterfering: cefotiam, cefadroxil

Interfering: cefazolin

KEY WORDS

plasma; column-switching; cefoperazone is IS

REFERENCE

Demotes-Mainard,F.; Vinçon,G.; Jarry,C.; Necciari,J.; Albin,H. Micromethod for the determination of cefpiramide in human plasma and urine by high-performance liquid chromatography using automated column switching, *J.Chromatogr.*, **1987**, *419*, 388–395.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma, plasma water, or urine + 1 mL 10% trichloroacetic acid + 16 μg phenacetin, vortex for 1 min, centrifuge at 3000 rpm for 12 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: Novapak C18

Mobile phase: MeOH:10 mM pH 5.5 KH_2PO_4 13:83 (sic)

Detector: UV 254

CHROMATOGRAM

Internal standard: phenacetin

Limit of quantitation: 1 μg/mL

KEY WORDS

plasma; plasma water; pharmacokinetics

REFERENCE

Hu,O.Y.-P.; Tang,H.-S.; Chang,C.-L. The influence of chronic lobular hepatitis on pharmacokinetics of cefoperazone—a novel galactose single-point method as a measure of residual liver function, *Biopharm.Drug Dispos.*, **1994**, *15*, 563–576.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 1 mL 10% trichloroacetic acid + 16 μg phenacetin, vortex for 1 min, centrifuge at 3000 rpm for 12 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: Novapak C18
Mobile phase: MeOH:10 mM pH 5.5 KH_2PO_4 13:83
Detector: UV 254

CHROMATOGRAM
Internal standard: phenacetin
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Hu,O.Y.; Tang,H.S.; Chang,C.L. Novel galactose single point method as a measure of residual liver function: example of cefoperazone kinetics in patients with liver cirrhosis, *J.Clin.Pharmacol.*, **1995**, *35*, 250–258.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 18
Limit of quantitation: 1680 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: cecal contents
Sample preparation: Dilute cecal contents in 2 mL phosphate buffered saline, centrifuge at 1500 g for 10 min. 500 μL Sample + 500 μL MeCN, vortex for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μL of the supernatant and add it to 3 mL dichloromethane, mix for 30 s, centrifuge at 1200 g for 5 min, inject a 20 μL aliquot of the upper aqueous phase.

HPLC VARIABLES
Column: 100 × 3 5 μm Hypersil ODS
Mobile phase: MeCN: 5 mM pH 5.5 acetate buffer 0.7:99.3
Flow rate: 1

Injection volume: 20
Detector: UV 254

OTHER SUBSTANCES
Also analyzed: ceftazidime, ceftriaxone

KEY WORDS
mouse; pharmacokinetics

REFERENCE
van Ogtrop,M.L.; Guiot,H.F.L.; Mattie,H.; van Furth,R. Modulation of the intestinal flora of mice by parenteral treatment with broad-spectrum cephalosporins, *Antimicrob.Agents Chemother.*, **1991**, *35*, 976–982.

SAMPLE
Matrix: cecal contents
Sample preparation: Weigh contents of cecum, dilute with 2 mL PBS, centrifuge at 1500 g for 10 min. Add a 500 μL aliquot of supernatant to 500 μL MeCN, mix on a whirlmixer for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μL of the supernatant and add it to 3 mL dichloromethane, mix for 30 s, centrifuge at 1200 g for 5 min, inject a 20 μL aliquot of the upper aqueous phase.

HPLC VARIABLES
Column: 100 × 3 5 μm Hypersil ODS
Mobile phase: MeCN:5 mM pH 5.5 acetate buffer 0.7:99.3
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Also analyzed: ceftazidime, ceftriaxone, cefepime

KEY WORDS
mouse

REFERENCE
van Ogtrop,M.L.; Guiot,H.F.L.; Mattie,H.; van Furth,R. Modulation of the intestinal flora of mice by parenteral treatment with broad-spectrum cephalosporins, *Antimicrob.Agents Chemother.*, **1991**, *35*, 976–982.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:10 mM pH 5.2 ammonium acetate 15:85
Flow rate: 1
Injection volume: 75
Detector: UV 254

CHROMATOGRAM
Retention time: 14.8
Internal standard: Cefuroxime
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate, adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 47

OTHER SUBSTANCES
Also analyzed: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefazolin, cefotaxime, ceftazidime, cephalosporin C

REFERENCE
Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 LiChrosorb RP-18
Mobile phase: MeOH:pH 4.55 acetate buffer 23:77
Flow rate: 1.5
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 5
Internal standard: diprophylline (10)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Jelinska,A.; Zajac,M. Effect of amino acids and amines on the stability of cefoperazone, *Pharmazie*, **1996**, *51*, 162–164.

SAMPLE
Matrix: solutions

Sample preparation: Add 100 μL solution to 1 mL 1 mg/mL aztreonam in water, vortex for 15 s, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C8
Mobile phase: MeCN:10 mM acetate buffer 28:72 containing 1.7 g/L tetrabutylammonium hydrogen sulfate, pH adjusted to 3.5 with 5 M NaOH (The buffer was 240 mL 10 mM sodium acetate and 480 mL 10 mM acetic acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.4
Internal standard: aztreonam (6.2)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
5% dextrose; saline

REFERENCE
Marble,D.A.; Bosso,J.A.; Townsend,R.J. Compatibility of clindamycin phosphate with aztreonam in polypropylene syringes and with cefoperazone sodium, cefonicid sodium, and cefuroxime sodium in partial-fill glass bottles, *Drug Intell.Clin.Pharm.*, **1988**, *22*, 54–57.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 22:78
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: penicillin G, methicillin, cephalothin

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Retention time: 9
Limit of detection: 60 nM

OTHER SUBSTANCES

Simultaneous: cefoxitin, ceftazidime, cefuroxime, cephalexin, cephradine

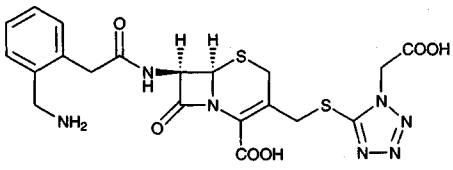
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Ceforanide



Molecular formula: $C_{20}H_{21}N_7O_6S_2$
Molecular weight: 519.56
CAS Registry No.: 60925-61-3
Merck Index: 1982
Lednicer No.: 3 214

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 50 μL 1 mg/mL sulfacetamide + 100 μL 6% trichloroacetic acid, vortex for 1 min, add 1 mL MeCN, centrifuge at 2500 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex for 1 min, shake for 10 min, centrifuge, inject a 100 μL aliquot of the aqueous phase. Analyze for free ceforanide by centrifuging serum at 3000 rpm for 20 min through an Amicon micropartition system with YMT membranes, 200 μL ultrafiltrate + 20 μL 1 mg/mL sulfacetamide, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 mm long C18 (Alltech)
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 10:90
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Internal standard: sulfacetamide

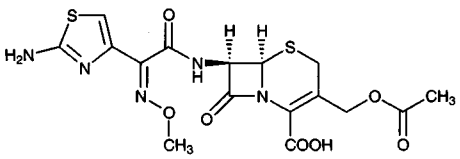
KEY WORDS

plasma; pharmacokinetics

REFERENCE

DiPiro,J.T.; Bayoumi,S.M.; Vallner,J.J.; Nesbit,R.R.; Gokhale,R.; Rissing,J.P. Intraoperative ceforanide pharmacokinetics and protein binding, *Antimicrob.Agents Chemother.*, **1985**, *27*, 487–490.

Cefotaxime



Molecular formula: $C_{16}H_{17}N_5O_7S_2$
Molecular weight: 455.47
CAS Registry No.: 63527-52-6, 64485-93-4 (sodium salt)
Merck Index: 1983
Lednicer No.: 3 216

SAMPLE

Matrix: aqueous humor, blood
Sample preparation: Aqueous humor. Inject a 10 μL aliquot directly. Plasma. Condition a 3 mL C18 SPE cartridge (Varian) with two 3 mL portions of MeCN and 3 mL buffer. Add 2 mL 625 ng/mL ciprofloxacin in buffer to 500 μL of plasma, mix, add to the SPE cartridge. Wash with 3 mL buffer. Remove moisture with vacuum (200 mbar) for 10 min. Elute with two 500 μL portions of MeCN:buffer 40:60. Vortex the eluate, inject a 10 μL aliquot. (Buffer was 100 mM Tris adjusted to pH 5.0 with HCl).

HPLC VARIABLES

Column: 300 × 4.6 5 μm endcapped ODS-Hypersil
Mobile phase: MeCN:DMF:10 mM NaH_2PO_4 15:6:79, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 285

CHROMATOGRAM

Retention time: 6.7
Internal standard: ciprofloxacin (12.0)
Limit of detection: 80 ng/mL (aqueous humor), 310 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites, ofloxacin

KEY WORDS

plasma; SPE

REFERENCE

Kraemer,H.-J.; Gehrke,R.; Breithaupt,A.; Breithaupt,H. Simultaneous quantification of cefotaxime, desacetylcefotaxime, ofloxacine and ciprofloxacine in ocular aqueous humor and in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 147–153.

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Plasma. 50 μL Plasma + 50 μL MeCN, mix for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Urine. Mix 200 μL MeCN and 100 μL urine for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot. Tissue. Weight out finely chopped tissue and suspend it in 200 μL water, sonicate for 60 s. Add 200 μL MeCN, vortex for 30 s, centrifuge at 10000 g for 15 min. Inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard C18 (Alltech)
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:50 mM pH 5.0 sodium dihydrogen phosphate 10:90
Flow rate: 1.0
Detector: UV 215

CHROMATOGRAM
Retention time: 11.6
Internal standard: cefotaxime

OTHER SUBSTANCES
Extracted: ampicillin

KEY WORDS
cefotaxime is IS; plasma; muscle; rat

REFERENCE
Cross,S.E.; Thompson,M.J.; Roberts,M.S. Distribution of systemically administered ampicillin, benzylpenicillin, and flucloxacillin in excisional wounds in diabetic and normal rats and effects of local topical vasodilator treatment, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1703–1710.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

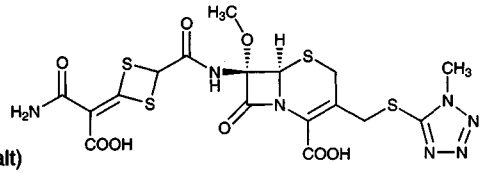
HPLC VARIABLES
Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Simultaneous: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefazolin, cefoperazone, ceftazidime, cephalosporin C

REFERENCE
Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

Cefotetan



Molecular formula: $C_{17}H_{17}N_7O_8S_4$
Molecular weight: 575.63
CAS Registry No.: 69712-56-7, 74356-00-6 (disodium salt)
Merck Index: 1984
Lednicer No.: 4 191, 192

SAMPLE

Matrix: blister fluid, blood
Sample preparation: Serum. 0.5 mL Serum + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to to 5 mL dichloromethane, vortex, centrifuge at 3000 g for 5 min, inject an aliquot of the upper layer. Blister fluid. Inject an aliquot directly.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: 100 mM phosphate buffer adjusted to pH 3.0 (?, no organic component reported)
Flow rate: 2
Detector: UV 229

CHROMATOGRAM

Retention time: 6
Limit of detection: 300 ng/mL

KEY WORDS

serum

REFERENCE

Jaresko,G.S.; Barriere,S.L.; Johnson,B.L.,Jr. Serum and blister fluid pharmacokinetics and bactericidal activities of ampicillin-sulbactam, cefotetan, cefoxitin, ceftizoxime, and ticarcillin-clavulanate [published erratum appears in Antimicrob Agents Chemother 1993 Mar;37(3):618], *Antimicrob.Agents Chemother.*, **1992**, *36*, 2233–2238.

SAMPLE

Matrix: blood, urine
Sample preparation: 200 μL Plasma + 200 μL 100 mM NaH_2PO_4 + 400 μL MeCN, mix for 5 s, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 2 mL dichloromethane, mix for 5 min, centrifuge at 4800 g for 10 min, inject a 5-50 μL aliquot of the upper aqueous phase. Urine. Centrifuge urine at 4800 g for 10 min, dilute 1:10 with 50 mM NaH_2PO_4, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:water 7.5:92.5 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 20 mg/L tetrabutylammonium bromide, pH 6.4 (plasma) or MeCN: water 5:95 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 22.5 mg/L tetrabutylammonium bromide, pH 6.4 (urine)
Flow rate: 1
Injection volume: 5-50
Detector: UV 280

CHROMATOGRAM

Retention time: 6.36 (plasma, epimer A), 6.84 (plasma, epimer B), 13.09 (urine, epimer A), 14.40 (urine, epimer B)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: iothalamic acid (UV 254)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kees,F.; Grobecker,H.; Naber,K.G. High-performance liquid chromatographic analysis of cefotetan epimers in human plasma and urine, *J.Chromatogr.*, **1984**, *305*, 363–371.

SAMPLE
Matrix: blood, urine
Sample preparation: Precipitate proteins with MeCN.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:70 mM sodium acetate 44:56, pH adjusted to 5.7 with acetic acid
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Internal standard: cefotetan
Limit of detection: 600 ng/mL

OTHER SUBSTANCES
Simultaneous: ceftriaxone

KEY WORDS
plasma; cefotetan is IS

REFERENCE
Paradis,D.; Vallée,F.; Allard,S.; Bisson,C.; Daviau,N.; Drapeau,C.; Auger,F.; LeBel,M. Comparative study of pharmacokinetics and serum bactericidal activities of cefpirome, ceftazidime, ceftriaxone, imipenem, and ciprofloxacin, *Antimicrob.Agents Chemother.*, **1992**, *36*, 2085–2092.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 8 4 μm Radial-Pak NOVA C18
Mobile phase: MeCN:water:acetic acid 20:80:2 containing 5 mM sodium 1 -hexanesulfonate
Flow rate: 2
Injection volume: 20
Detector: UV 264

CHROMATOGRAM
Retention time: 2.6
Internal standard: 3,5-dinitrobenzoic acid (10.2)

OTHER SUBSTANCES
Simultaneous: cefmetazole, cefodizime, cefoperazone, cefotaxime, piperacillin

REFERENCE
Marunaka,T.; Matsushima,E.; Maniwa,M. Determination of cefodizime in biological materials by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 329–339.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

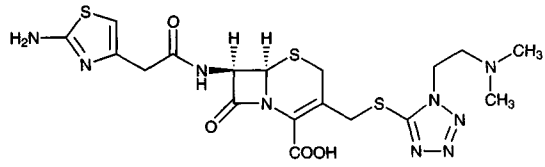
HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate + 10 mM tetrabutylammonium bromide + 1% acetic acid 30:70
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 290

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefotiam



Molecular formula: $C_{18}H_{23}N_9O_4S_3$
Molecular weight: 525.64
CAS Registry No.: 61622-34-2, 66309-69-1 (HCl)
Merck Index: 1985
Lednicer No.: 3 215

SAMPLE

Matrix: blister fluid, blood
Sample preparation: 200 µL Serum or blister fluid + 200 µL 50 mM pH 6.2 sodium phosphate + 400 µL MeCN, mix, add 2 mL dichloromethane, extract, inject a 50 µL aliquot of the aqueous supernatant. Refrigerate samples before injection.

HPLC VARIABLES

Column: 125 × 4.6 5 µm Spherisorb ODS-2
Mobile phase: MeCN:water:acetic acid 80:1000:2, adjust pH to 5.1 with 10 M NaOH
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 5.4
Limit of detection: 20 ng/mL (serum), 10 ng/mL (blister fluid)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Kees,F.; Raasch,W.; Steger,M.; Grobecker,H. High-performance liquid chromatographic assay for cefotiam and d3-cefotiam in human serum, *J.Chromatogr.*, **1990**, *525*, 484–489.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 100 µL 1 mg/mL cefotaxime in water + 1 mL MeCN, vortex for 3 s, centrifuge for 5 min. Remove the upper layer and add it to 3 mL dichloromethane, shake for 5 min, centrifuge, inject a 20 µL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 7 µm Eicompak MA-ODS (Eicom Corp.)
Mobile phase: MeCN:10 mM pH 4.2 acetate buffer 15:85
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.3
Internal standard: cefotaxime (4.1)
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Chiba,K.; Tsuchiya,M.; Kato,J.; Ochi,K.; Kawa,Z.; Ishizaki,T. Cefotiam disposition in markedly obese athlete patients, Japanese sumo wrestlers, *Antimicrob.Agents Chemother.*, **1989**, *33*, 1188–1192.

SAMPLE
Matrix: blood
Sample preparation: Dilute plasma 1:5 with 50 mM pH 7.7 phosphate buffer, inject a 500 μL aliquot onto column A with mobile phase A, after 10 min backflush the contents of column A onto column B with mobile phase C, after 5 min remove column A from the circuit and continue to elute column B with mobile phase C, monitor the effluent from column B. Wash column A with mobile phase B for 10 min then re-equilibrate column A with mobile phase A for 10 min.

HPLC VARIABLES
Column: A Guard Pak μBondapak C18; B 150 × 4.6 5 μm YMC ODS A-302 (Yamamura Chemical)
Mobile phase: A 50 mM pH 7.7 phosphate buffer; B MeCN:water 60:40; C MeCN:50 mM pH 7.7 phosphate buffer 12:88
Column temperature: 25
Flow rate: 1
Injection volume: 500
Detector: UV 254

CHROMATOGRAM
Retention time: 18
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; column-switching

REFERENCE
Yamashita,K.; Motohashi,M.; Yashiki,T. Automated high-performance liquid chromatographic method for the simultaneous determination of cefotiam and delta3-cefotiam in human plasma using column switching, *J.Chromatogr.*, **1992**, *577*, 174–179.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 1 mL 10 (serum) or 100 (urine) μg/mL ceftezole in MeOH, shake on a microthermomixer for 30 s, filter (urine samples only), centrifuge at 3000 rpm for 3 min, filter (0.5 μm) the supernatant, dilute with two volumes of water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 Nucleosil 5C18
Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:100 mM pH 4.4 acetate buffer 5:95 (Buffer was 100 mM acetic acid: 100 mM sodium acetate 2:1.)
Flow rate: 0.8
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: ceftezole (11)
Limit of detection: 2000 ng/mL (urine), 100 ng/mL (serum)

KEY WORDS
serum

REFERENCE
Itakura,K.; Mitani,M.; Aoki,I.; Usui,Y. High performance liquid chromatographic assay of cefsulodin, cefotiam and cefmenoxime in serum and urine, *Chem.Pharm.Bull.(Tokyo)*, **1982**, *30*, 622–627.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 20 μL 0.45 N phosphoric acid + 100 μL methanol + 20 μL 270 μmol/L cephalexin, vortex 15 s, centrifuge for 3 min, remove 100 μL supernatant, inject 20 μL. Urine. 10 μL Urine + 0.5 mL water + 20 μL 270 μmol/L cephalexin, vortex 15 s, remove 100 μL supernatant, inject 20 μL.

HPLC VARIABLES
Guard column: 100 × 4.7 Co:Pell ODS
Column: 150 × 4.7 LiChrosorb RP-18
Mobile phase: MeOH: 20 mM tetrabutylammonium hydrogen sulfate and 24 mM K_3PO_4 and 16 mM KH_2PO_4 23:77
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 24
Internal standard: Cephalexin
Limit of detection: 50 nmol/mL (urine), 1 nmol/mL (plasma)

OTHER SUBSTANCES
Simultaneous: cefuroxime

KEY WORDS
plasma

REFERENCE
Lecaillon,J.B.; Rouan,M.C.; Souppart,C.; Febvre,N.; Juge,F. Determination of cefsulodin, cefotiam, cephalexin, cefotaxime, deacetylcefotaxime, cefuroxime and cefroxadin in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 257–267.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 260

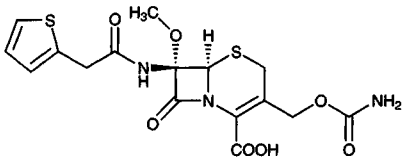
OTHER SUBSTANCES

Also analyzed: cefpiramide, cefazolin, cefmenoxime, cefbuperazone, cefoxitin, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefoxitin



Molecular formula: $C_{16}H_{17}N_3O_7S_2$
Molecular weight: 427.46
CAS Registry No.: 35607-66-0, 33564-30-6 (sodium salt)
Merck Index: 1986
Lednicer No.: 2 435; 4 190

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 6.8 (mobile phase A), 8.0 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: Prepare an anion-exchange SPE cartridge in a 6 mL syringe barrel with a filter paper disc in the bottom. Pack with DEAE-A-25 Sephadex in PBS to a bed volume of 3 mL, wash with PBS, place filter paper on top. Add 500 μL serum to SPE cartridge, add 500 μL PBS to SPE cartridge, wash with 4 mL PBS, elute with 5 mL 1 M NaCl, inject a 100 μL aliquot of the eluate. (PBS was 8 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KCl, and 0.2 g KH_2PO_4 in 1 L water, pH 7.2.)

HPLC VARIABLES

Column: 300 × 4 10 μm octadecylsilane
Mobile phase: MeCN:buffer 13:87 (Buffer was water adjusted to pH 2.8 with acetic acid, about 1.5 mL/min.)
Flow rate: 1.5

Injection volume: 100
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Extracted: cephapirin
Noninterfering: amikacin, amphotericin B, azathioprine, carbenicillin, chloral hydrate, cimetidine, dopamine, fluphenazine, furosemide, hydrochlorothiazide, insulin, levothyroxine, methylprednisolone, nitroglycerin, oxacillin, prednisone, procainamide, sulfamethoxazole, tolazamide, tolbutamide, triamterene, trimethoprim
Interfering: cefotaxime

KEY WORDS
serum; SPE

REFERENCE
Fasching,C.E.; Peterson,L.R. Anion-exchange extraction of cephapirin, cefotaxime, and cefoxitin from serum for liquid chromatography, *Antimicrob.Agents Chemother.*, **1982**, *21*, 628–633.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.2
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 3 μg/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefotaxime, cephapirin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood

Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 11.52:0.48:88, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5
Internal standard: cefoperazone (7.5)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: cefotaxime
Interfering: cephaloridine, cephalexin

KEY WORDS

plasma

REFERENCE

Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE

Matrix: blood
Sample preparation: 350 μL Serum + 150 μL 150 μg/mL temocillin in water + 250 μL 400 mM HCl + 3.5 mL chloroform:n-amyl alcohol (3:1), mix for 5 min, centrifuge for 5 min. Remove the organic layer and add it to 350 μL 10 mM pH 7.0 phosphate buffer, mix for 5 min, centrifuge for 5 min, inject a 20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 100 mM ammonium acetate adjusted to pH 4.0 with glacial acetic acid.)
Flow rate: 1.8
Injection volume: 20
Detector: UV 242

CHROMATOGRAM

Retention time: 7.4
Internal standard: temocillin (5.4)

OTHER SUBSTANCES

Extracted: cefuroxime, cephalothin, ticarcillin
Noninterfering: acetaminophen, acetazolamide, allopurinol, amikacin, ampicillin, azlocillin, caffeine, cefamandole, cefoperazone, cefotaxime, cefsulodin, ceftazidime, ceftizoxime, chloramphenicol, chlorpromazine, clindamycin, dicloxacillin, 5-fluorocytosine, flurazepam, gentamicin, methicillin, metronidazole, mezlocillin, moxalactam, nafcillin, penicillin, phenobarbital, piperacillin, procainamide, rifampin, sulfamethoxazole, theophylline, thienamycin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum

REFERENCE
Shull,V.H.; Dick,J.D. Determination of ticarcillin levels in serum by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1985**, *28*, 597–600.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL plasma + 300 μL 5 μg/mL cefotaxime in pH 3.5 10 mM acetate buffer and keep at 4°. Inject 100 μL onto column A with mobile phase A. After 5 min backflush column A with mobile phase B onto column B for 3 min. Re-equilibrate column A with mobile phase A for 16 min.

HPLC VARIABLES
Column: A 40 × 2 37-50 μm Corasil RP C18; B 20 × 4 25-40 μm Lichrosorb RP-8 + 250 × 4 Partisil ODS-3
Mobile phase: A 10 mM pH 3.5 acetate buffer; B MeCN:20 mM pH 4.3 acetate buffer 15:85
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 12.2
Internal standard: cefotaxime
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: cephalexin, cefuroxime, cephaloridine
Noninterfering: cefotiam, cefadroxil, cefazolin, cefoperazone, cephalothin, cefamandole, aspirin, diclofenac, alclofenac, lonazolac, piroxicam, ibuprofen, indomethacin, ketoprofen, naproxen, phenylbutazone, mefenamic acid, caffeine

KEY WORDS
plasma; column-switching; rat; human

REFERENCE
Lee,Y.J.; Lee,H.S. Simultaneous determination of cefoxitin, cefuroxime, cephalexin and cephaloridine in plasma using HPLC and a column-switching technique, *Chromatographia*, **1990**, *30*, 80–84.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Add 25 μL 25 μg/mL cephalothin to 500 μL serum, vortex, place sample in an ultrafree MC filter unit with a 10000 MW cutoff (Millipore), centrifuge at 16000 g for 30 min, inject a 180 μL aliquot of the filtrate. Tissue. Homogenize 100 mg tissue in 1 mL water using a Polytron homogenizer (Brinkman), add 25 μL 25 μg/mL cephalothin, vortex, centrifuge at 1000 g for 15 min, filter (Acrodisc CR PTFE 0.2 μm filter, prewet with water and MeOH) the supernatant, inject a 180 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 100 × 8 10 μm μBondapak C18
Mobile phase: MeCN:5 mM KH_2PO_4:glacial acetic acid 22:77.5:0.5
Flow rate: 2
Injection volume: 180
Detector: UV 235

CHROMATOGRAM
Retention time: 8.75 (serum), 8.55 (tissue)

Internal standard: cephalothin (13.55 (serum), 13.95 (tissue))
Limit of detection: 10 ng/mL (serum), 50 ng/mL (tissue)

KEY WORDS
cat; colon; serum

REFERENCE
Cox,S.K.; Burnette,J.D.; Huss,B.T.; Frazier,D. Determination of cefoxitin in serum and tissue, *J.Chromatogr.B*, **1998**, *705*, 145–148.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 1 g tissue with 10 mL pH 6.0 100 mM phosphate buffer (Polytron homogenizer), centrifuge at 3000 g for 10 min. 0.5 mL Serum or tissue homogenate supernatant + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex , centrifuge at 3000 g for 5 min, inject a 50 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 300 mm long μBondapak C18
Mobile phase: MeCN:100 mM sodium phosphate 14:86, adjust pH to 6.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Also analyzed: cefoperazone

KEY WORDS
serum

REFERENCE
Bawdon,R.E.; Hemsell,D.L.; Guss,S.P. Comparison of cefoperazone and cefoxitin concentrations in serum and pelvic tissue of abdominal hysterectomy patients, *Antimicrob.Agents Chemother.*, **1982**, *22*, 999–1003.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. Add 500 μL MeOH to 500 μL serum, vortex for 30 s, centrifuge at 10000 g for 15 min, inject a 50 μL aliquot of the supernatant. Urine. Add 4.5 mL 50 mM pH 6.0 potassium phosphate buffer to 500 μL urine, mix vigorously for 15 s, filter 1 mL of this solution (0.45 μm filter, Millex HA, Millipore), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Nucleosil-5 C18
Column: 150 × 4.6 5 μm Nucleosil-5 C18
Mobile phase: A MeCN:100 mM pH 6.0 potassium phosphate buffer 13:87; B MeCN:100 mM pH 6.0 potassium phosphate buffer 20:80 containing 0.1 mM hexadecyltrimethylammonium
Flow rate: 1
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Retention time: 8.5 (A), 8.0 (B)
Limit of quantitation: 200 ng/mL (serum), 2 μg/mL (urine)

KEY WORDS
serum

REFERENCE
García-Glez,J.C.; Méndez,R.; Martín-Villacorta,J. Quantitative determination of semisynthetic cephamycins in human serum and urine by ion-exchange, reversed-phase and ion-pair chromatography, *J.Chromatogr.A*, **1998**, *812*, 197–204.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 1650 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak C18
Mobile phase: MeOH:5 mM pH 7.5 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Simultaneous: degradation products, cefazolin

KEY WORDS
injections; water; stability-indicating

REFERENCE
Stiles,M.L.; Tu,Y.H.; Allen,L.V.,Jr. Stability of cefazolin sodium, cefoxitin sodium, ceftazidime, and penicillin G sodium in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1989**, *46*, 1408–1412.

SAMPLE
Matrix: formulations
Sample preparation: Mix an aliquot with an equal volume of 5 mg/mL cefoxitin, dilute with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolv (Waters)
Mobile phase: MeCN:buffer 18:86 (Buffer was 2.46 g anhydrous sodium acetate, 8 mL glacial acetic acid, and 200 mg tetrabutylammonium hydrogen sulfate in 1 L water, pH 3.0.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.0
Internal standard: cefoxitin

OTHER SUBSTANCES
Simultaneous: cefotaxime, metronidazole

KEY WORDS
injections; saline; cefoxitin is IS

REFERENCE
Belliveau,P.P.; Nightingale,C.H.; Quintiliani,R. Stability of cefotaxime sodium and metronidazole in 0.9% sodium chloride injection or in ready-to-use metronidazole bags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1561–1563.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:50 mM KH_2PO_4 7:93
Flow rate: 3
Detector: UV 273

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: cefazolin

REFERENCE
Allababidi,S.; Shah,J.C. Efficacy and pharmacokinetics of site-specific cefazolin delivery using biodegradable implants in the prevention of post-operative wound infections, *Pharm.Res.*, **1998**, *15*, 325–333.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm ODS-Hypersil
Mobile phase: MeCN:10 mM ammonium acetate 10:90

Flow rate: 2
Injection volume: 25
Detector: UV 280

REFERENCE

Eley,A.; Greenwood,D. Beta-lactamases of type culture strains of the *Bacteroides fragilis* group and of strains that hydrolse cefoxitin, latamoxef and imipenem, *J.Med.Microbiol.*, **1986**, *21*, 49–57.

SAMPLE

Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

OTHER SUBSTANCES

Also analyzed: cefpiramide, cefazolin, cefmenoxime, cefbuperazone, cefotiam, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 60 nM

OTHER SUBSTANCES

Simultaneous: cefoperazone, ceftazidime, cefuroxime, cephalexin, cephradine

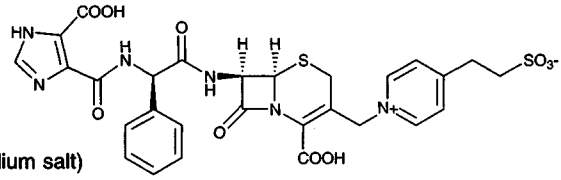
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Cefpimizole



Molecular formula: $C_{28}H_{26}N_6O_{10}S_2$
Molecular weight: 670.68
CAS Registry No.: 84880-03-5, 85287-61-2 (sodium salt)
Merck Index: 1988
Lednicer No.: 4 185

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 1 mL buffer + 4 mL MeCN, mix, let stand at least 2 h at 4°, centrifuge, remove the supernatant, wash the precipitate with 2 mL MeCN:buffer 75:25, centrifuge. Combine the supernatants and add them to 200 μL dichloromethane, let stand at -20°. Remove the aqueous phase and add it to 50 μL 200 μg/mL acetophenone in MeOH, mix, filter (0.45 μm, Gelman Acrodisc-CR), store at 4°, inject an aliquot. Urine. 100 μL Urine + 4 mL 10 μg/mL acetophenone in mobile phase, mix, filter (0.45 μm, Gelman Acrodisc-CR), store at 4°, inject an aliquot. (Buffer was 10 mM EDTA and 50 mM tetrabutylammonium hydroxide, pH 5.0.)

HPLC VARIABLES

Guard column: 50 × 2.1 35 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:buffer 140:260, pH adjusted to 6.0 with glacial acetic acid (Buffer was 40 mL 100 mM EDTA, 50 mL 400 mM tetrabutylammonium hydroxide, and 2510 mL water.)
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Internal standard: acetophenone (18)
Limit of detection: 50 ng/mL (plasma), 1000 ng/mL (urine)
Limit of quantitation: 330 ng/mL (plasma), 16800 ng/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Lakings,D.B.; Wozniak,J.M. High-performance liquid chromatographic methods for the determination of cefpimizole in plasma and urine, *J.Chromatogr.*, **1984**, *308*, 261–271.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeCN and 3 mL buffer. Homogenize 250 mg rat tissue with 2.5 mL water and 0.5 mL 100 mM EDTA in a small tissue grinder (Ace Glass), add a 100 μL aliquot to the SPE cartridge, wash with 3 mL water, wash with 2 mL MeCN:water 10:90, elute with 2 mL MeCN:water 30:70, add 10 μg acetophenone to the eluate, mix, filter (0.45 μm, Gelman Acrodisc CR), store at 4°, inject an aliquot. (Buffer was 10 mM EDTA and 50 mM tetrabutylammonium hydroxide, pH 5.)

HPLC VARIABLES

Guard column: 50 × 2.1 35 μm Co:Pell ODS
Column: 250 × 4.5 5 μm C18 (IBM)

Mobile phase: MeOH:water 33:67 containing 1 mM EDTA and 5 mM tetrabutylammonium hydroxide, adjusted to pH 6.5 with acetic acid
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 14
Internal standard: acetophenone (21)
Limit of detection: 120000 ng/g

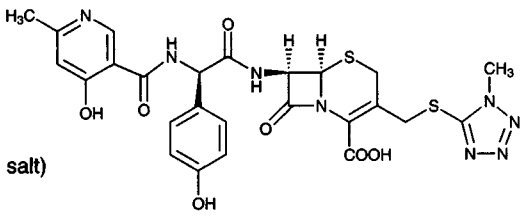
KEY WORDS

rat; liver; spleen; kidney; SPE

REFERENCE

Friis,J.M.; Lakings,D.B. High-performance liquid chromatographic method for the determination of cefpimizole in tissue, *J.Chromatogr.*, **1986**, *382*, 399–404.

Cefpiramide



Molecular formula: $C_{25}H_{24}N_8O_7S_2$
Molecular weight: 612.65
CAS Registry No.: 70797-11-4, 74849-93-7 (sodium salt)
Merck Index: 1989
Lednicer No.: 4 188

SAMPLE

Matrix: bile, blood, duodenal fluid, tissue, urine
Sample preparation: Serum, bile, duodenal fluid. 500 μL Serum, bile or duodenal fluid + 500 μL MeCN, centrifuge. Remove the supernatant and add it to 3.5 mL dichloromethane, centrifuge, inject a 20 μL aliquot of the supernatant. Urine. Dilute urine 1:10 with MeOH, inject an aliquot. Tissue. Wash gallbladder wall with pH 7 phosphate buffer, dry, weigh, homogenize in 1 mL pH 7 phosphate buffer, filter, microfilter, inject an aliquot of the microfiltrate.

HPLC VARIABLES

Column: reverse phase
Mobile phase: MeCN:water:200 mM ammonium acetate 15:75:10, adjust pH to 5.0 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Limit of detection: 1000 ng/mL (urine), 500 ng/mL (bile), 50 ng/mL (serum, duodenal juice)

KEY WORDS

serum; gallbladder wall; pharmacokinetics

REFERENCE

Brogard,J.M.; Jehl,F.; Adloff,M.; Blickle,J.F.; Monteil,H. High hepatic excretion in humans of cefpiramide, a new cephalosporin, *Antimicrob.Agents Chemother.*, **1988**, *32*, 1360–1364.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Dilute urine and bile with water as necessary. 50 μL Plasma, diluted urine, or diluted bile + 50 μL 10% perchloric acid + 50 μL 3-butylxanthine in phosphate buffer, mix, centrifuge at 11000 g for 10 min. Add the supernatant to 240 μL 1 M sodium acetate, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeOH:30 mM pH 5.0 isotonic phosphate buffer 20:80
Column temperature: 40
Flow rate: 1.5
Detector: UV 279

CHROMATOGRAM

Internal standard: 3-butylxanthine

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE
Muraoka,I.; Hasegawa,T.; Nadai,M.; Wang,L.; Haghgoo,S.; Tagaya,O.; Nabeshima,T. Biliary and renal excretions of cefpiramide in Eisai hyperbilirubinemic rats, *Antimicrob.Agents Chemother.*, **1995**, *39*, 70–74.

SAMPLE
Matrix: blood
Sample preparation: Filter plasma (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm GFF-S5-80 internal-surface reversed phase "Pinkerton" (Regis)
Mobile phase: THF:100 mM potassium phosphate 2.5:97.5, pH 7.0
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 8.4

KEY WORDS
plasma; direct injection

REFERENCE
Nakagawa,T.; Shibukawa,A.; Shimono,N.; Kawashima,T.; Tanaka,H.; Haginaka,J. Retention properties of internal-surface reversed-phase silica packing and recovery of drugs from human plasma, *J.Chromatogr.*, **1987**, *420*, 297–311.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 300 μL Serum + 300 μL cold (iced) 38.5 μg/mL cefamandole in MeOH:100 mM pH 5.2 sodium acetate 80:20, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot. Urine. 100 μL Urine + 1 mL 76.9 μg/mL cefamandole in MeOH, vortex for 30 s, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm C18 (Waters)
Mobile phase: MeCN:MeOH:100 mM sodium acetate:water 14.4:0.6:10:75, pH 5.2
Flow rate: 2
Injection volume: 5-10
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: cefamandole (9.5)
Limit of detection: 12850 ng/mL (urine), 920 ng/mL (serum)

OTHER SUBSTANCES
Noninterfering: metronidazole, vancomycin, nafcillin, ticarcillin, clindamycin, gentamicin

KEY WORDS
serum

REFERENCE
Conte,J.E.,Jr.; Zurlinden,E. Column liquid chromatographic determination of cefpiramide in human serum and urine, *J.Chromatogr.*, **1987**, *417*, 452–457.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μL Plasma + 100 μL 10 μg/mL cefoperazone in 100 mM pH 5 ammonium acetate, vortex for 15 s, centrifuge at 8700 g for 2 min, inject a 20-200 μL aliquot onto column A with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent. Re-equilibrate column A with mobile phase A before the next injection.. Urine. Dilute urine with saline. 100 μL Diluted urine + 500 μL 10 μg/mL cefoperazone in 100 mM pH 5 ammonium acetate, inject a 20-200 μL aliquot onto column A with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent. Re-equilibrate column A with mobile phase A before the next injection.

HPLC VARIABLES

Column: A 20 × 4 38-50 μm Corasil C18; B 100 × 8 10 μm μBondapak C18

Mobile phase: A water:triethylamine 1000:4 adjusted to pH 3.0 with orthophosphoric acid; B MeCN:water:triethylamine 750:250:4, pH adjusted to 3.0 with orthophosphoric acid

Flow rate: A 2; B 3.8

Injection volume: 20-200

Detector: UV 270

CHROMATOGRAM

Retention time: 2.33

Internal standard: cefoperazone (3.66)

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: ceftazidime, ceftriaxone, cefotaxime, cephaloridine, ceforanide, moxalactam, cefazolin, cefonicid, cephalothin

Noninterfering: cefotiam, cefadroxil

KEY WORDS

plasma; column-switching

REFERENCE

Demotes-Mainard,F.; Vinçon,G.; Jarry,C.; Necciari,J.; Albin,H. Micromethod for the determination of cefpiramide in human plasma and urine by high-performance liquid chromatography using automated column switching, *J.Chromatogr.*, **1987**, *419*, 388–395.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 ODS-80TM (Tosoh)

Mobile phase: MeCN:buffer 20:80 (Buffer was 10 mM tetrabutylammonium bromide and 10 mM ammonium acetate.)

Detector: UV 240

REFERENCE

Tamai,I.; Maekawa,T.; Tsuji,A. Membrane potential-dependent and carrier-mediated transport of cefpiramide, a cephalosporin antibiotic, in canalicular rat liver plasma membrane vesicles, *J.Pharmacol.Exp.Ther.*, **1990**, *253*, 537–544.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

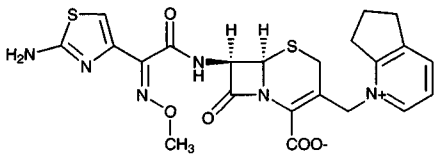
OTHER SUBSTANCES

Also analyzed: cefazolin, cefmenoxime, cefbuperazone, cefoxitin, cefotiam, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefpirome



Molecular formula: $C_{22}H_{22}N_6O_5S_2$
Molecular weight: 514.59
CAS Registry No.: 84957-29-9, 98753-19-6 (sulfate)
Merck Index: 1990
Lednicer No.: 5 158

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL cefpirome sulfate solution + 100 μL 10% trichloroacetic acid, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:50 mM pH 3.5-4.0 ammonium phosphate buffer 8:92
Flow rate: 1.2
Injection volume: 20
Detector: UV 257

CHROMATOGRAM
Internal standard: cefpirome

OTHER SUBSTANCES
Extracted: ceftazidime

KEY WORDS
cefpirome is IS; serum

REFERENCE
Klepser,M.E.; Patel,K.B.; Nicolau,D.P.; Quintiliani,R.; Nightingale,C.H. Comparison of the bactericidal activities of ofloxacin and ciprofloxacin alone and in combination with ceftazidime and piperacillin against clinical strains of *Pseudomonas aeruginosa*, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2503–2510.

SAMPLE
Matrix: blood, urine
Sample preparation: Add aminophylline, precipitate proteins with MeCN, delipidate with dichloromethane.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:100 mM sodium acetate 14:86, pH adjusted to 4.2 with acetic acid
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8
Internal standard: aminophylline
Limit of detection: 100 ng/mL

KEY WORDS
plasma

REFERENCE

Paradis,D.; Vallée,F.; Allard,S.; Bisson,C.; Daviau,N.; Drapeau,C.; Auger,F.; LeBel,M. Comparative study of pharmacokinetics and serum bactericidal activities of cefpirome, ceftazidime, ceftriaxone, imipenem, and ciprofloxacin, *Antimicrob.Agents Chemother.*, **1992**, *36*, 2085–2092.

SAMPLE

Matrix: cell suspensions
Sample preparation: Filter (0.45 μm).

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere IP ion pair
Mobile phase: MeOH:100 mM sodium perchlorate adjusted to pH 2.5 with concentrated sulfuric acid 35:65
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 3.8

OTHER SUBSTANCES

Extracted: carumonam (UV 295), ceftriaxone, cefotaxime

REFERENCE

Bellido,F.; Pechère,J.-C.; Hancock,R.E.W. Novel method for measurement of outer membrane permeability to new β-lactams in intact *Enterobacter cloacae* cells, *Antimicrob.Agents Chemother.*, **1991**, *35*, 68–72.

SAMPLE

Matrix: milk, urine
Sample preparation: Milk. 50 μL Milk + 200 μL 200 μg/mL β-hydroxypropyltheophylline in isopropanol:water 80:20, vortex for 20 s, centrifuge at 2000 g at 4° for 2 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 200 μL mobile phase, vortex for 20 s, centrifuge at 2000 g at 4° for 2 min, inject a 10 μL aliquot. Urine. Centrifuge at 1500 g at 4° for 5 min. Remove 50 μL of the supernatant and add it to 200 μL 200 μg/mL β-hydroxypropyltheophylline in mobile phase, vortex for 15 s, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 μm ODS (Hewlett-Packard)
Mobile phase: MeOH:buffer 12:88 (milk) or 10:90 (urine) (Buffer was 0.3% triethylamine in water adjusted to pH 5.1 with glacial acetic acid.)
Column temperature: 50
Flow rate: 0.5
Injection volume: 10-20
Detector: UV 240

CHROMATOGRAM

Retention time: 2.0 (milk), 2.8 (urine)
Internal standard: β-hydroxypropyltheophylline (4.0 (milk), 5.0 (urine))
Limit of detection: 625 ng/mL

OTHER SUBSTANCES

Noninterfering: ampicillin, tobramycin, gentamicin, amikacin, ticarcillin, aspirin, acetaminophen, ibuprofen, theophylline, caffeine, chlorpheniramine, cimetidine, carbamazepine, phenytoin, phenobarbital

REFERENCE

Kearns,G.L.; Johnson,V.A.; Hendry,I.R.; Wells,T.G. Microanalytical high-performance liquid chromatographic assay for cefpirome in human milk and urine, *J.Chromatogr.*, **1992**, *574*, 356–360.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 Nucleosil 10C18
Mobile phase: MeCN:1% KH_2PO_4 1:8
Flow rate: 1
Injection volume: 20
Detector: UV (wavelength not specified)

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Sugioka,T.; Asano,T.; Chikaraishi,Y.; Suzuki,E.; Sano,A.; Kuriki,T.; Shirotsuka,M.; Saito,K. Stability and degradation pattern of cefpirome (HR 810) in aqueous solution, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 1998–2002.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:phosphate buffer 8:92
Flow rate: 1.2

OTHER SUBSTANCES

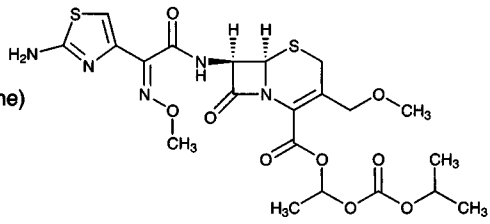
Simultaneous: ceftazidime

REFERENCE

Nicolau,D.P.; Nightingale,C.H.; Banevicius,M.A.; Fu,Q.; Quintiliani,R. Serum bactericidal activity of ceftazidime: Continuous infusion versus intermittent injections, *Antimicrob.Agents Chemother.*, **1996**, *40*, 61–64.

Cefpodoxime proxetil

Molecular formula: $C_{21}H_{27}N_5O_9S_2$
Molecular weight: 557.61
CAS Registry No.: 87239-81-4, 80210-62-4 (cefpodoxime)
Merck Index: 1991
Lednicer No.: 5 158



SAMPLE

Matrix: blood
Sample preparation: Condition a C8 SPE cartridge with 1 mL MeOH:DMF 90:10 and 1 mL 1% phosphoric acid, do not allow to go dry. 200 µL Plasma + 1 mL 1 µg/mL cefaclor in 1% phosphoric acid + 200 µL MeCN:1% phosphoric acid 1:99, add to the SPE cartridge, wash with 1 mL MeOH:1% phosphoric acid 5:95, wash with 500 µL 1% phosphoric acid, elute the contents of the SPE cartridge onto the analytical column with the mobile phase.

HPLC VARIABLES

Guard column: 12 × 4.6 7 µm Newguard C8
Column: 250 × 4.6 5 µm IB-SIL C18 (Phenomenex)
Mobile phase: MeCN:MeOH:50 mM pH 6.0 sodium acetate buffer 4:4:92 (After elution of IS inject 1 mL MeCN:water 90:10 to remove late eluting peaks.)
Flow rate: 2
Detector: UV 254

CHROMATOGRAM

Retention time: 10.8
Internal standard: cefaclor (17)
Limit of detection: 3 ng/mL
Limit of quantitation: 11 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, cefotaxime
Noninterfering: acetaminophen, amikacin, ceftazidime, ceftriaxone, gentamicin, nafcillin, phenytoin, ticarcillin, tobramycin, vancomycin
Interfering: theophylline

KEY WORDS

SPE; plasma

REFERENCE

Steenwyk,R.C.; Brewer,J.E.; Royer,M.E.; Cathcart,K.S. Reversed-phase liquid chromatographic determination of cefpodoxime in human plasma, *J.Liq.Chromatogr.*, **1991**, *14*, 3641–3656.

SAMPLE

Matrix: blood, ear fluid
Sample preparation: 50 µL Plasma or ear effusion + 50 µL 40 µg/mL cefuroxime in water + 2 mL MeCN, vortex briefly, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 75 µL mobile phase, inject a 25 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 2.1 5 µm Hypersil C18
Column: 250 × 2.1 5 µm Hypersil C18

Mobile phase: MeCN:buffer 7.5:92.5 After elution of IS increase ratio to 50:50 to clean column. (Buffer was 25 mM acetate and 15 mM triethylamine adjusted to pH 4.3 with NaOH.)
Column temperature: 40
Flow rate: 0.35
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5
Internal standard: cefuroxime (5.9)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; chinchilla; middle ear effusion; pharmacokinetics

REFERENCE
Lovdahl,M.J.; Reher,K.E.; Russlie,H.Q.; Canafax,D.M. Determination of cefpodoxime levels in chinchilla middle ear fluid and plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *653*, 227–232.

SAMPLE
Matrix: blood, sinus mucosa
Sample preparation: Plasma. Condition a 3 mL 500 mg Bond Elut C8 SPE cartridge with 3 mL MeOH and 2 mL 1% phosphoric acid. 500 μL Plasma + 1 mL 2 μg/mL cefaclor in 1% phosphoric acid, mix, add to the SPE cartridge, wash with 3 mL 1% perchloric acid, elute with 750 μL MeOH, inject a 50 μL aliquot of the eluate. Sinus mucosa. Condition a 1 mL Bond Elut C8 SPE cartridge with 1 mL MeOH and 1 mL 1% phosphoric acid. Chop sample with a scalpel, weigh out 20 mg and add it to 500 μL 10 mM pH 7.0 phosphate buffer, rotate at 4° for 12 h, centrifuge at 800 g for 10 min. 400 μL Supernatant + 1 mL 50 ng/mL cefaclor, mix, add to the SPE cartridge, wash with 1% perchloric acid, elute with 150 μL MeOH, inject a 75 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm C18 (Shandon)
Column: 250 × 4.6 5 μm Supelcosil LC 18
Mobile phase: MeOH:MeCN:50 mM pH 3.8 acetate buffer 10:3:87 (plasma) or 12:2:86 (sinus mucosa)
Flow rate: 1
Injection volume: 50-75
Detector: UV 235

CHROMATOGRAM
Retention time: 16.8
Internal standard: cefaclor (18.2)
Limit of detection: 10 ng/mL (plasma)
Limit of quantitation: 130 ng/g (sinus mucosa), 50 ng/mL (plasma)

KEY WORDS
plasma; SPE

REFERENCE
Camus,F.; Deslandes,A.; Harcouet,L.; Farinotti,R. High-performance liquid chromatographic method for the determination of cefpodoxime levels in plasma and sinus mucosa, *J.Chromatogr.B*, **1994**, *656*, 383–388.

SAMPLE
Matrix: blood, tissue, urine

Sample preparation: Serum. 500 μL Serum + 500 μL MeCN, vortex, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 3.2 mL dichloromethane, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min, inject a 20 μL aliquot of the upper aqueous layer. Urine. Dilute urine 1:10 with water, inject an aliquot. Tissue. Grind frozen under liquid nitrogen, extract with 3 mL pH 7 phosphate buffer at 4° for 12 h, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 70 × 4.6 3 μm Ultrasphere XL-ODS
Mobile phase: MeCN:21.5 mM ammonium acetate 7:93, adjusted to pH 5 with glacial acetic acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.6
Limit of detection: 300 ng/mL (urine), 20 ng/mL (serum)

OTHER SUBSTANCES

Noninterfering: aminoglycosides, amoxicillin, ampicillin, aztreonam, carbamazepine, cefadroxil, cefixime, cefotaxime, cefpiramide, ceftazidime, digitoxin, furosemide, lidocaine, phenobarbital, quinidine, quinolones, salicylic acid, theophylline

KEY WORDS

serum; kidney

REFERENCE

Molina,F.; Jehl,F.; Gallion,C.; Penner,F.; Monteil,H. Determination of the third generation oral cephalosporin cefpodoxime in biological fluids by high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *563*, 205–210.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.628 (peak 1), 18.945 (peak 2)

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.45 μm) urine, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A. After 10 min elute the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeOH: water for 2 min then re-equilibrate column A with mobile phase A for 6 min.

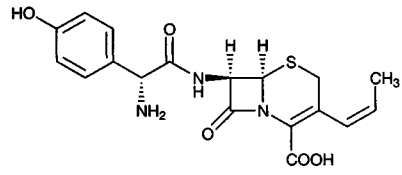
HPLC VARIABLES
Column: A 30 × 4.6 5 μm C18 (Brownlee) (Condition a new column with 30 mL MeOH and 30 mL MeOH:water 50:50.); B 250 × 4.6 5 μm IB-SIL C18 (Phenomenex)
Mobile phase: A MeOH:0.2% pH 2.0 phosphoric acid 10:90; B MeCN:50 mM pH 5.2 sodium acetate buffer 7:93
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 19
Limit of detection: 34 ng/mL
Limit of quantitation: 110 ng/mL

KEY WORDS
column-switching; pharmacokinetics

REFERENCE
Bombardt,P.A.; Cathcart,K.S.; Bothwell,B.E.; Closson,S.K. Determination of cefpodoxime levels and cefpodoxime stability in human urine by direct injection HPLC with column-switching, *J.Liq.Chromatogr.*, **1991**, *14*, 1729–1746.

Cefprozil



Molecular formula: $C_{18}H_{19}N_3O_5S$
Molecular weight: 389.43
CAS Registry No.: 92665-29-7, 121123-17-9 (monohydrate)
Merck Index: 1992
Lednicer No.: 5 158

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μL water + 50 μL 25 μg/mL cefprozil + 100 μL 1 M hydrochloric acid, vortex briefly. Filter (Centrifree micropartition unit) 1 mL mixture while centrifuging at 2000 g for 10 min. Inject a 25 μL aliquot of the ultrafiltrate. Urine. 250 μL urine + 500 μL 250 μg/mL cefprozil + 4.25 mL 10 mM pH 3.5 acetate buffer, vortex briefly. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleogil C18 (plasma) or 125 × 4.6 5 μm Lichrosorb C18 (urine)
Mobile phase: MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 8:92 (plasma) or MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 10:90 (urine)
Flow rate: 1.2 (plasma), 1.0 (urine)
Injection volume: 25
Detector: UV 260

CHROMATOGRAM

Retention time: 9.2 (plasma), 6.3 (urine)
Internal standard: cefprozil

OTHER SUBSTANCES

Extracted: cephalexin

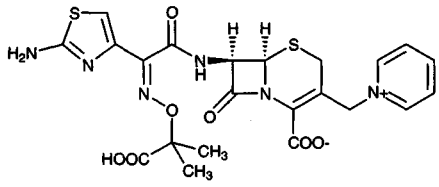
KEY WORDS

plasma; cefprozil is IS

REFERENCE

Barbhaiya,R.H. A pharmacokinetic comparison of cefadroxil and cephalexin after administration of 250, 500 and 1000 mg solution doses, *Biopharm.Drug Dispos.*, **1996**, *17*, 319–330.

Ceftazidime



Molecular formula: $C_{22}H_{22}N_6O_7S_2$
Molecular weight: 546.58
CAS Registry No.: 72558-82-8, 78439-06-2 (pentahydrate)
Merck Index: 1995
Lednicer No.: 4 192

SAMPLE

Matrix: blood, CSF
Sample preparation: Deproteinize serum or CSF with MeCN, centrifuge, add the supernatant to dichloromethane, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: reversed-phase
Mobile phase: MeCN:100 mM pH 5.0 NaH_2PO_4 buffer 8:92 containing 5 mM pentanesulfonic acid
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 94 ng/mL (serum), 67 ng/mL (CSF)

KEY WORDS

serum; pharmacokinetics

REFERENCE

Nau,R.; Prange,H.W.; Kinzig,M.; Frank,A.; Dressel,A.; Scholz,P.; Kolenda,H.; Sörgel,F. Cerebrospinal fluid ceftazidime kinetics in patients with external ventriculostomies, *Antimicrob.Agents Chemother.*, **1996**, *40*, 763–766.

SAMPLE

Matrix: formulations
Sample preparation: Reconstitute ceftazidime injection in sodium carbonate with 10 mL 0.9% NaCl, agitate vigorously, dilute with 0.9% NaCl injection to obtain a ceftazidime concentration of 60 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC18
Mobile phase: MeCN:100 mM pH 7.0 K_3PO_4 buffer 6:94
Flow rate: 1
Injection volume: 20
Detector: UV 257

CHROMATOGRAM

Retention time: 8.4
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, pyridine

KEY WORDS

injections; stability-indicating

REFERENCE

Stendal,T.L.; Klem,W.; Tonnesen,H.H.; Kjonniksen,I. Drug stability and pyridine generation in ceftazidime injection stored in an elastomeric infusion device, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 683–685.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 1.73

OTHER SUBSTANCES

Simultaneous: granisetron (UV 300)

KEY WORDS

stability-indicating; injections; saline

REFERENCE

Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 39.5

OTHER SUBSTANCES

Simultaneous: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefazolin, cefoperazone, cefotaxime, cephalosporin C

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C-6
Mobile phase: MeCN:50 mM sodium acetate containing 100 mM acetic acid 6:94 (A) or 4:96 (B)
Flow rate: 1.4
Injection volume: 20
Detector: UV 258

CHROMATOGRAM
Retention time: 5.3 (A), 7.2 (B)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Fubara,J.O.; Notari,R.E. A kinetic oxymoron: Concentration-dependent first-order rate constants for hydrolysis of ceftazidime, *J.Pharm.Sci.*, **1998**, *87*, 53–58.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 100 × 4.6 4 μm Novapak C18
Mobile phase: MeCN:7.5 mM pH 7.0 phosphate buffer 40:60 containing 2 g/L tetrabutylammonium phosphate
Flow rate: 1.5
Injection volume: 10
Detector: UV 242

CHROMATOGRAM
Retention time: 1.6
Internal standard: ceftriaxone

REFERENCE
Plumridge,R.J.; Rieck,A.M.; Annus,T.P.; Langton,S.R. Stability of ceftriaxone sodium in polypropylene syringes at -20, 4, and 20°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2320–2323.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 1.5
Limit of detection: 60 nM

OTHER SUBSTANCES
Simultaneous: cefoperazone, cefoxitin, cefuroxime, cephalexin, cephradine

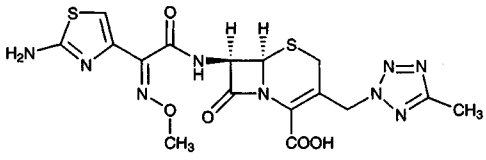
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Cefteram



Molecular formula: $C_{16}H_{17}N_9O_5S_2$
Molecular weight: 479.50
CAS Registry No.: 82547-58-8
Merck Index: 1996

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 3 mL MeOH and 3 mL buffer. 1 mL Plasma + 50 μL 500 μg/mL penicillin G (sodium salt) in MeOH + 2 mL buffer, vortex, add to SPE cartridge, wash with buffer, elute with 500 μL MeOH:10 mM pH 5.2 potassium phosphate buffer 90:10, inject a 25 μL aliquot. (Buffer was 121 g Trizma base in 1 L water, adjust pH to 7.0 with concentrated HCl. Dilute 1:100 to obtain the 10 mM buffer.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeOH:10 mM pH 5.2 potassium phosphate buffer 20:80 (Buffer was 1 M KH_2PO_4 adjusted to pH 5.2 with 5 M KOH, dilute 1:100 with water to give working buffer.)
Column temperature: 50
Flow rate: 0.9
Injection volume: 25
Detector: UV 225

CHROMATOGRAM

Retention time: 4.6
Internal standard: penicillin G (12)
Limit of quantitation: 59.8 ng/mL

KEY WORDS

plasma; SPE; method stated to be applicable to urine (no details)

REFERENCE

Hicks,C.M.; Powell,M.L. Rapid analysis of ceftetrame in human plasma using sorbent extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *497*, 349–354.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 5 μm YMC Pack AMC312 ODS (Yamamura Chemical)
Mobile phase: MeCN:water:60% perchloric acid:sodium perchlorate monohydrate 156:844:1:5 (v/v/v/w)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.6
Internal standard: cefteram

OTHER SUBSTANCES

Extracted: E-1100

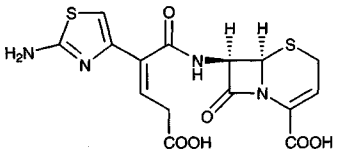
KEY WORDS

cefteram is IS; extraction of cefteram from plasma is not demonstrated

REFERENCE

Tokumura,T.; Horie,T. Determination of a novel β-lactam antibiotic (E-1100) in rat plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *620*, 153–157.

Ceftibuten



Molecular formula: $C_{15}H_{14}N_4O_6S_2$
Molecular weight: 410.43
CAS Registry No.: 97519-39-6
Merck Index: 1998
Lednicer No.: 5 160

SAMPLE

Matrix: blood
Sample preparation: Filter plasma (0.45 μm). Inject 50 μL plasma onto column A with mobile phase A, after 6 min the contents of column A were back-flushed onto column B with mobile phase B, after 3 min column A was removed from the circuit and column B was eluted with mobile phase B. Column A was washed with mobile phase C for 5 min then equilibrated with mobile phase A (1.5 mL/min) for 11 min until next injection.

HPLC VARIABLES

Column: A 35 × 4.6 20 μm TSK BSA-ODS; B 150 × 4.6 5 μm Nucleosil 5C18
Mobile phase: A MeOH:5 mM tetrabutylammonium bromide + 2 mM $(NH_4)H_2PO_4$, pH 5.0 1:50; B MeCN:MeOH:10 mM tetrabutylammonium bromide + 2 mM $(NH_4)H_2PO_4$, pH 5.0 6:3:25; C MeCN:5 mM tetrabutylammonium bromide + 10 mM $(NH_4)H_2PO_4$, pH 7.0 3:10
Flow rate: A 1.2; B 1; C 1.5
Injection volume: 50
Detector: UV 256

CHROMATOGRAM

Retention time: 22.9 (cis), 20.8 (trans)
Limit of detection: 100 ng/mL

KEY WORDS

plasma; column-switching

REFERENCE

Matsuura,A.; Nagayama,T.; Kitagawa,T. Analytical studies on β-lactam antibiotics. III. Automated high-performance liquid chromatographic method for the determination of the orally active antibiotic ceftibuten in human plasma and urine, *J.Chromatogr.*, **1989**, *494*, 231–245.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 50 μL 100 mM ammonium acetate, mix, inject a 100 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 4 min elute column A onto column B with mobile phase A, after 2 min elute column B with mobile phase B. At the end of each day change the guard column and wash column A with mobile phase B overnight.

HPLC VARIABLES

Column: A guard column (unspecified) + 150 × 3.9 μBondapak phenyl; B 300 × 4.6 μBondapak phenyl
Mobile phase: A 100 mM pH 6.5 ammonium acetate; B MeCN:100 mM pH 6.5 ammonium acetate 2:98
Flow rate: 1
Injection volume: 100
Detector: UV 263

CHROMATOGRAM

Retention time: 13 (cis only)

Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Pan,H.-T.; Kumari,P.; Lim,J.; Lin,C.-C. Determination of a cephalosporin antibiotic, ceftibuten, in human plasma with column-switching high-performance liquid chromatography with ultraviolet detection, *J.Pharm.Sci.*, **1992**, *81*, 663–666.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 10 μL ceftazidime solution, homogenize, add 500 μL MeCN, vortex for 10 s, centrifuge at 3000 g for 10 min. Remove 800 μL of the supernatant and ad it to 5 mL dichloromethane, vortex for 10 s, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Spherisorb C8
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:buffer 4.5:95.5 (Buffer was 3.85 g/L ammonium acetate + 2 mL triethylamine, adjusted to pH 4 with formic acid.)
Column temperature: 50
Flow rate: 1.75
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.8 (cis), 9.8 (trans)
Internal standard: ceftazidime (19.8)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: norfloxacin, pefloxacin, ofloxacin, amikacin, tobramycin, acyclovir

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Kinowski,J.M.; Bressolle,F.; Fabre,D.; Goncalves,F.; Rouzier-Panis,R.; Galtier,M. High-performance liquid chromatographic determination of ceftibuten and its metabolite in biological fluids: applications in pharmacokinetic studies, *J.Pharm.Sci.*, **1994**, *83*, 736–741.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL 50 μg/mL acyclovir in water + 100 μL 200 mM pH 7 sodium phosphate buffer, mix well, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak CN guard-PAK
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:50 mM ammonium acetate 2:98
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 11.0 (ceftibuten), 13.4 (ceftibuten-trans)
Internal standard: acyclovir (18)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, amoxicillin, ampicillin, aspirin, aztreonam, caffeine, cefamandole, cefotiam, cefsulodin, ceftazidime, ceftriaxone, cefuroxime, cephaloridine, cephalothin, chlorpheniramine, gentamicin, moxolactam, nafcillin, piperacillin, pseudoephedrine, theophylline, ticarcillin, vancomycin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lim,J.M.; Kim,H.; Marco,A.; Mojaverian,P.; Lin,C.-C. Liquid chromatographic determination of ceftibuten, a new oral cephalosporin, in human plasma and urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 699–703.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 10 μg/mL cefadroxil + 1 mL MeCN, vortex, centrifuge at 2000 g for 10 min. Remove the aqueous phase and add it to 2.5 mL dichloromethane, vortex, centrifuge, inject a 25 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm C18 (Waters)
Mobile phase: MeCN:150 mM ammonium acetate 0.7:99.3, pH 7.0
Flow rate: 1.1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Internal standard: cefadroxil
Limit of detection: 400 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
serum; mouse; pharmacokinetics

REFERENCE
Onyeji,C.O.; Nicolau,D.P.; Nightingale,C.H.; Quintiliani,R. Optimal times above MICs of ceftibuten and cefaclor in experimental intra-abdominal infections, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1112–1117.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: 100 μL Plasma, urine, or dialysate + 100 μL 200 mM pH 7 phosphate buffer + acyclovir, inject a 5 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM ammonium acetate 2:98
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Internal standard: acyclovir
Limit of quantitation: 500 ng/mL (urine), 100 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics; cis and trans isomers separated

REFERENCE
Kelloway,J.S.; Awni,W.M.; Lin,C.C.; Lim,J.; Affrime,M.B.; Keane,W.F.; Matzke,G.R.; Halstenson,C.E. Pharmacokinetics of ceftibuten-*cis* and its *trans* metabolite in healthy volunteers and in patients with chronic renal insufficiency, *Antimicrob.Agents Chemother.*, **1991**, *35*, 2267–2274.

SAMPLE
Matrix: blood, ultrafiltrate
Sample preparation: Denature with EtOH (if necessary), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 10 C18
Mobile phase: MeCN:MeOH:PIC A 6:3:50 (PIC A is tetrabutylammonium phosphate (Waters).)
Flow rate: 1.2
Injection volume: 15
Detector: UV 256

CHROMATOGRAM
Retention time: 10.7 (cis), 12.3 (trans)
Limit of quantitation: 100 ng/mL

KEY WORDS
serum; isomerization

REFERENCE
Shimada,J.; Hori,S.; Oguma,T.; Yoshikawa,T.; Yamamoto,S.; Nishikawa,T.; Yamada,H. Effects of protein binding on the isomerization of ceftibuten, *J.Pharm.Sci.*, **1993**, *82*, 461–465.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL 200 mM pH 7.0 sodium phosphate, vortex, allow to sit for 15 min, add 20 μL 100 μg/mL acyclovir, add 800 μL MeCN, vortex for 20 s, centrifuge at 2500 g at 25° for 2 min. Remove the supernatant and add it to 1.6 mL dichloromethane, vortex for 20 s, centrifuge at 2500 g at 25° for 1 min, inject an aliquot of the organic layer. Urine. Dilute urine samples 10-20-fold with water, treat with Nonidet P-40 detergent, let stand for 5 min, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:50 mM ammonium acetate 2:98
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 262 (plasma), UV 254 (urine)

CHROMATOGRAM
Internal standard: acyclovir
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kearns,G.L.; Reed,M.D.; Jacobs,R.F.; Ardite,M.; Yogev,R.D.; Blumer,J.L. Single-dose pharmacokinetics of ceftibuten (SCH 39720) in infants and children, *Antimicrob.Agents Chemother.*, **1991**, *35*, 2078–2084.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Spherisorb 5 ODS2
Mobile phase: MeOH:10% acetic acid 25:75 or MeOH:5 mM heptanesulfonic acid 25:75
Flow rate: 1.7
Detector: UV 264

CHROMATOGRAM
Limit of detection: 250 ng/mL

KEY WORDS
pharmacokinetics; cis and trans isomers separated

REFERENCE
Wise,R.; Nye,K.; O'Neill,P.; Wostenholme,M.; Andrews,J.M. Pharmacokinetics and tissue penetration of ceftibuten, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1053–1055.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ODS Hitachi 3053
Mobile phase: MeCN:buffer 10:90 (Buffer was 50 mM citric acid and 100 mM KCl, pH 2.5.)
Flow rate: 0.7
Detector: UV 262

REFERENCE
Naasani,I.; Sugawara,M.; Kobayashi,M.; Iseki,K.; Miyazaki,K. Transport mechanism of ceftibuten, a dianionic cephem, in rat renal brush-border membrane, *Pharm.Res.*, **1995**, *12*, 605–608.

SAMPLE
Matrix: sputum
Sample preparation: Sputum + 200 μL 100 mM Ammonium acetate, vortex, centrifuge at 9000 g for 10 min, inject a 100 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 4 min elute column A onto column B with mobile phase A, after 2 min elute column B with mobile phase B. At the end of each day change the guard column and wash column A with mobile phase B overnight.

HPLC VARIABLES
Column: A guard column (unspecified) + 150 × 3.9 μBondapak phenyl; B 300 × 4.6 μBondapak phenyl
Mobile phase: A 100 mM ammonium acetate; B MeCN:100 mM ammonium acetate 2:98
Flow rate: 1
Injection volume: 100
Detector: MS, Vestec model 201 LC/MS, thermospray, T1 170°, probe tip 225°, block 312°, vapor 270°; monitor m/z 226

CHROMATOGRAM
Retention time: 8 (cis only, after start of elution with mobile phase B)
Limit of quantitation: 500 ng/mL

KEY WORDS
column-switching; LC-MS

REFERENCE
Pan,H.-T.; Kumari,P.; de Silva,J.A.F.; Lin,C.-C. Determination of ceftibuten in sputum by column-switching high-performance liquid chromatography on-line with thermospray mass spectrometry, *J.Pharm.Sci.*, **1993**, *82*, 52–55.

SAMPLE
Matrix: urine
Sample preparation: Dilute 1 mL urine to 10 mL with pH 7.0 phosphate buffer, filter (0.45 μm). Inject 20 μL onto column A with mobile phase A, after 1.5 min the contents of column A were forward-flushed onto column B with mobile phase B, after another 2 min column A was removed from the circuit and column B was eluted with mobile phase B. Column A was washed with mobile phase A (1.5 mL/min) for at least 10 min until next injection.

HPLC VARIABLES
Column: A 50 × 4.6 5 μm Cosmosil 5C18; B 150 × 4.6 5 μm Nucleosil 5C18
Mobile phase: A MeOH:10 mM $(NH_4)H_2PO_4$, pH 5.0 1:10; B MeCN:MeOH:5 mM tetrabutylammonium bromide + 5 mM tetraamylammonium bromide + 8 mM $(NH_4)H_2PO_4$, pH 5.0 25:10:65
Flow rate: A 1; B 1
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 20.0 (cis), 18.6 (trans)
Limit of detection: 1000 ng/mL

KEY WORDS
column-switching

REFERENCE
Matsuura,A.; Nagayama,T.; Kitagawa,T. Analytical studies on β-lactam antibiotics. III. Automated high-performance liquid chromatographic method for the determination of the orally active antibiotic ceftibuten in human plasma and urine, *J.Chromatogr.*, **1989**, *494*, 231–245.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 1/10 to 1/40 with water, inject a 50 μL aliquot onto column A with mobile phase A, after 5 min elute the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 2 min.

HPLC VARIABLES
Column: A 30 × 4.6 5 μm Spheri 5 amino; B 20 × 4.6 10 μm Spherisorb C8 + 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: A 30 mM NaH_2PO_4; B MeCN:buffer 2.5:97.5 (Buffer was 17.9 g/L Na_2HPO_4 adjusted to pH 7 with 10% phosphoric acid.)
Column temperature: 50
Flow rate: A 0.6; B 1

Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 6 (cis), 7 (trans)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: norfloxacin, pefloxacin, ofloxacin, amikacin, tobramycin, acyclovir

KEY WORDS

column-switching; pharmacokinetics

REFERENCE

Kinowski,J.M.; Bressolle,F.; Fabre,D.; Goncalves,F.; Rouzier-Panis,R.; Galtier,M. High-performance liquid chromatographic determination of ceftibuten and its metabolite in biological fluids: applications in pharmacokinetic studies, *J.Pharm.Sci.*, **1994**, *83*, 736–741.

SAMPLE

Matrix: urine
Sample preparation: Urine. 100 μL urine + 200 μL 200 mM pH 7 sodium phosphate buffer, inject a 15 μL aliquot onto column A and elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Guard column: μBondapak CN guard-PAK
Column: A 30 × 4.6 Spheri-10 amino H2GU (Brownlee); B 250 × 4.6 Partisil 10 ODS-3
Mobile phase: A 30 mM $(NH_4)H_2PO_4$; B MeCN:50 mM pH 7 sodium phosphate buffer 2.5:97.5
Flow rate: A 0.6; B 1
Injection volume: 15
Detector: UV 254

CHROMATOGRAM

Retention time: 12.3 (ceftibuten), 13.7 (ceftibuten-trans)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Noninterfering: aztreonam, cefmenoxime, cefoxitin, cefotiam, ceftriaxone, cephalexin

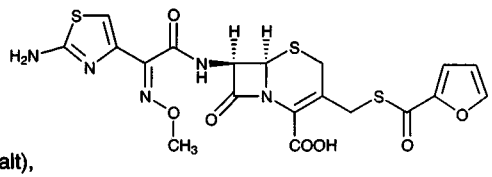
KEY WORDS

column-switching

REFERENCE

Lim,J.M.; Kim,H.; Marco,A.; Mojaverian,P.; Lin,C.-C. Liquid chromatographic determination of ceftibuten, a new oral cephalosporin, in human plasma and urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 699–703.

Ceftiofur



Molecular formula: $C_{19}H_{17}N_5O_7S_3$
Molecular weight: 523.57
CAS Registry No.: 80370-57-6, 104010-37-9 (sodium salt), 103980-44-5 (HCl)
Merck Index: 1999
Lednicer No.: 4 187

SAMPLE

Matrix: blood, milk
Sample preparation: 500 μL Serum or milk + 500 μL MeCN:water 50:50, vortex for 10-15 s, filter while centrifuging (Amicon Centricon-10, 10000 daltons cut-off) at 4000 g for 30 min, inject a 10-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 3 μm Ultremex phenyl
Mobile phase: MeCN:buffer 20:80 (Buffer was 0.25% 80% phosphoric acid, 0.25% triethylamine, 2.5 mM sodium octanesulfonate, and 2.5 mM sodium decanesulfonate in water.)
Column temperature: 40
Flow rate: 0.8-1
Injection volume: 10-100
Detector: UV 265.8

CHROMATOGRAM

Retention time: 12.5
Limit of detection: 50 ppb

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; cow

REFERENCE

Tyczkowska,K.L.; Voyksner,R.D.; Anderson,K.L.; Aronson,A.L. Determination of ceftiofur and its metabolite desfuroylceftiofur in bovine serum and milk by ion-paired liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 123–134.

SAMPLE

Matrix: milk
Sample preparation: Condition a 6 mL 1 g Varian Mega Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL 100 mM ammonium acetate. Dilute 2 g milk with 8 mL 100 mM ammonium acetate, shake by hand. Add to the SPE cartridge and apply vacuum to maintain flow rate at 1-2 drops/s. Rinse the tube twice with 5 mL portions of 100 mM ammonium acetate, add the rinses to the SPE cartridge, wash with 5 mL 100 mM ammonium acetate, dry the cartridge under vacuum. Elute quickly with 3 mL MeOH. Evaporate the eluate to ca. 1 mL under a stream of nitrogen. Dilute to 2 mL with 100 mM acetate buffer. Filter (0.2 μm) and inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18-DB
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:100 mM pH 3.3 acetate buffer 80:20 (Prepare buffer as follows. Mix 463 mL 200 mM acetic acid with 37 mL 200 mM sodium acetate and make up to 1 L with water.)

Column temperature: 35
Flow rate: 1.0
Injection volume: 100
Detector: UV 293

CHROMATOGRAM
Retention time: 12.0
Limit of detection: 4 ppb
Limit of quantitation: 7 ppb

OTHER SUBSTANCES
Simultaneous: chloramphenicol, erythromycin, gentamycin, novobiocin, penicillins, spectinomycin, sulfonamides, tetracyclines

KEY WORDS
SPE; cow

REFERENCE
McNeilly,P.J.; Reeves,V.B.; Deveau,E.J. Determination of ceftiofur in bovine milk by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 844–847.

SAMPLE
Matrix: milk
Sample preparation: Dilute 500 μL milk with 500 μL MeCN:water 50:50, vortex for 10-15 s, filter (Amicon microseparation system with 10000 molecular-mass cutoff filter (Centrion-10)) while centrifuging at 4000 g for 30 min. Inject a 100 μL aliquot of the colorless ultrafiltrate. (Protect from light!)

HPLC VARIABLES
Column: 75 × 3.9 Nova Pak C18
Mobile phase: Gradient. A was MeCN. B was 1% acetic acid containing 25 mM heptafluorobutyric acid. A:B from 5:95 to 95:5 over 9 min
Flow rate: 1
Injection volume: 100
Detector: MS, Hewlett-Packard 5989, electrospray, Hewlett-Packard API interface with hexapole ion guide, nebulizing gas nitrogen, 290°, SIM, m/z 524, post-column solvent addition of isopropanol:propionic acid 25:75

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 10 ppb
Limit of quantitation: 25 ppb

KEY WORDS
ultrafiltrate; protect from light

REFERENCE
Keever,J.; Voyksner,R.D.; Tyczkowska,K.L. Quantitative determination of ceftiofur in milk by liquid chromatography--electrospray mass spectrometry, *J.Chromatogr.A*, **1998**, *794*, 57–62.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with

MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 24.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 28:72 (Buffer was 3.3 mM phosphoric acid containing 6.7 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 290

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: 10 mL Milk + 2 mL 200 mM tetraethylammonium chloride, stir, slowly add 38 mL MeCN over 30 s, let stand for 5 min, decant the supernatant through a plug of glass wool. 40 mL Filtrate + 1 mL water, evaporate under reduced pressure to 1-2 mL, make up to 4 mL with water, filter (0.45 μm polyvinylidene difluoride). Inject 2 mL into an LC system (150 × 4.6 5 μm Supelcosil LC-18; MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 40:60 over 27 min, to 0:100 over 1 min; 1 mL/min; UV 210 and 295), collect a 1.5 mL fraction at retention time for ceftiofur (23 min), evaporate to 1 mL, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 29:71 (Buffer was 3.3 mM phosphoric acid and 6.7 mM KH_2PO_4.)
Flow rate: 1
Injection volume: 200
Detector: UV 295

CHROMATOGRAM
Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES
Also analyzed: ampicillin, amoxicillin, cephapirin, penicillin G, penicillin V, cloxacillin

KEY WORDS
cow

REFERENCE
Moats,W.A.; Harik-Khan,R. Liquid chromatographic determination of β-lactam antibiotics in milk: A multiresidue approach, *J.AOAC Int.*, **1995**, *78*, 49–54.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0

potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM

Retention time: 18.13

OTHER SUBSTANCES

Extracted: ampicillin, cephapirin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS

SPE

REFERENCE

Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 6 mL 1 g Mega Bond Elut C18 SPE cartridge with 4 mL MeOH and 5 mL phosphate buffer. Condition a 10 mL 500 mg Bond Elut LRC SAX SPE cartridge with 2 mL MeOH, 2 mL MeOH:100 mM NaCl 25:75, and two 1 mL portions of water. Condition a 10 mL 100 mg Bond Elut LRC SCX SPE cartridge with 1 mL MeOH, 2 mL MeOH:100 mM calcium chloride 25:75, and two 1 mL portions of water. Fat. Homogenize (Waring blender) 10 g fat, 20 mL 0.4% dithioerythritol in borate buffer, and 20 mL hexane at medium speed for 5 min, centrifuge at 3000 g for 10 min. Remove a 2 mL aliquot of the aqueous (bottom) layer and add it to 13 mL 0.4% dithioerythritol in borate buffer, shake at 50° for 15 min, add 3 mL 14% iodoacetamide in phosphate buffer, mix well, let stand at room temperature, adjust pH to 2.5-2.6 with 5% phosphoric acid, centrifuge at 4° at 48000 g for 20 min, add the supernatant to the C18 SPE cartridge, wash with 5 mL phosphate buffer, wash with 3 mL 10 mM NaOH, elute with 3 mL MeCN:water 15:85. Add the eluate to 15 mL water, add this mixture to the SAX SPE cartridge, wash with 1 mL water, elute with 2.5 mL MeCN:5% acetic acid 5:95, inject a 500 μL aliquot of the eluate. Muscle, liver kidney. Homogenize (Waring blender) 10 g tissue and 140 mL 0.4% dithioerythritol in borate buffer at medium speed for 5 min, centrifuge at 3000 g for 10 min. Remove a 15 mL aliquot of the homogenate and shake it at 50° for 15 min, add 3 mL 14% iodoacetamide in phosphate buffer, mix well, let stand at room temperature, adjust pH to 2.5-2.6 with 5% phosphoric acid, centrifuge at 4° at 48000 g for 20 min, add the supernatant to the C18 SPE cartridge, wash with 5 mL phosphate buffer, wash with 3 mL 10 mM NaOH, elute with 3 mL MeCN:water 15:85. Add the eluate to 15 mL water, add this mixture to the SAX SPE cartridge, wash with 1 mL water, elute with 2.5 mL MeCN:5% acetic acid 5:95. Add the eluate to 10 mL water, mix well, add the mixture to the SCX SPE cartridge, wash with 1 mL water, elute with 2.5 mL MeCN:100 mM NaCl 5:95 (muscle, kidney) or 2 mL MeCN:100 mM NaCl 10:90 (liver), inject a 500 μL aliquot of the eluate. (Borate buffer was 19 g sodium borate and 3.7 g KCl in 1 L water, pH 9. Phosphate buffer was 3.4 g KH_2PO_4 in 700 mL water, pH adjusted to 7 with KOH, made up to 1 L with water.)

HPLC VARIABLES

Guard column: BDS Hypersil C18
Column: 250 × 4.6 BDS Hypersil C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 65:35 over 35 min, wash with 50:50 at 1.5 mL/min for 15 min, re-equilibrate for 20 min (muscle, kidney) or A:B 85:15 for 5 min, to 75:25 over 5 min, wash with 50:50 at 1.5 mL/min for 15 min, re-equilibrate for 20 min (liver, fat).
Flow rate: 1
Injection volume: 500
Detector: UV 266

CHROMATOGRAM

Retention time: 7 (liver, fat), 26 (muscle, kidney)
Limit of detection: 10-30 ng/g
Limit of quantitation: 100 ng/g

OTHER SUBSTANCES

Extracted: cephapirin
Noninterfering: cefoperazone, cefquinome, cephacetril, dihydrostreptomycin, neomycin, penicillin G, spectinomycin, tetracycline

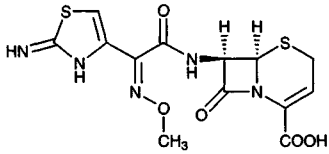
KEY WORDS

pig; muscle; kidney; liver; fat; derivatization; SPE; rugged

REFERENCE

Beconi-Barker,M.G.; Roof,R.D.; Millerioux,L.; Kausche,F.M.; Vidmar,T.J.; Smith,E.B.; Callahan,J.K.; Hubbard,V.L.; Smith,G.A.; Gilbertson,T.J. Determination of ceftiofur and its desfuroylceftiofur-related metabolites in swine tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *673*, 231–244.

Ceftizoxime



Molecular formula: $C_{13}H_{13}N_5O_5S_2$
Molecular weight: 383.41
CAS Registry No.: 68401-81-0, 68401-82-1 (sodium salt)
Merck Index: 2000
Lednicer No.: 3 218

SAMPLE
Matrix: blood
Sample preparation: After serum deproteination with MeCN, extract the serum samples with 1 mL dichloromethane. Inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:pH 7.0 phosphate buffer 25:75
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Internal standard: acetazolamide
Limit of quantitation: 250 ng/mL

KEY WORDS
serum

REFERENCE
Belliveau,P.P.; Freeman,C.D.; Nicolau,D.P.; Nightingale,C.H.; Tessier,P.R.; Quintiliani,R. Serum bactericidal activity of ceftizoxime and ceftriaxone against pathogens associated with community-acquired and nosocomial pneumonias, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1024–1027.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM
Retention time: 4.3 (mobile phase A), 4.9 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftriaxone, cefuroxime, cephaloridine, cephalothin

Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, teicoplanin, theophylline, vancomycin
Interfering: ranitidine

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 200 μg/mL cefotaxime in water + 1 mL MeCN, vortex for 5 s, centrifuge at 30 g for 5 min. Remove the supernatant and add it to 1.5 mL dichloromethane, vortex for 5 s, centrifuge for 5 min, inject a 10-20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Guard column: 50 mm long CO:PELL ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 13:84.2:2.8
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 310

CHROMATOGRAM
Retention time: 6
Internal standard: cefotaxime (9)
Limit of quantitation: 1500 ng/mL

OTHER SUBSTANCES
Simultaneous: ceftriaxone, cephalexin, moxalactam, cephaloridine, cefoxitin, cefamandole
Noninterfering: gentamicin, tobramycin, amikacin, clindamycin, erythromycin, vancomycin, penicillin, cefoperazone, piperacillin, ticarcillin, carbenicillin, apalcillin
Interfering: cefazolin, cephapirin

KEY WORDS
serum

REFERENCE
McCormick,E.M.; Echols,R.M.; Rosano,T.G. Liquid chromatographic assay of ceftizoxime in sera of normal and uremic patients, *Antimicrob.Agents Chemother.*, **1984**, *25*, 336–338.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 2 mL MeCN, vortex for 1 min, centrifuge at 4° at 3000 g for 5 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 30 s, centrifuge at 3000 g for 5 min, remove the upper aqueous layer and keep it at 4°, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Bondapak C18
Mobile phase: MeCN:100 mM pH 6.1 phosphate buffer 2:98
Injection volume: 20
Detector: UV 229

CHROMATOGRAM
Limit of quantitation: 100 ng/mL

KEY WORDS
serum

REFERENCE
Fortunato,S.J.; Bawdon,R.E.; Welt,S.I.; Swan,K.F. Steady-state cord and amniotic fluid ceftizoxime levels continuously surpass maternal levels, *Am.J.Obstet.Gynecol.*, **1988**, *159*, 570–573.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL ice-cold 50 μg/mL cefotaxime in MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, hold at -20° for 10 min, centrifuge at 1500 g for 10 min, inject 15 μL of supernatant.

HPLC VARIABLES
Guard column: 10 μm C18 Guard-PAK
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 100:876:24
Flow rate: 1.5
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Internal standard: cefotaxime
Limit of detection: 800 ng/mL

OTHER SUBSTANCES
Also analyzed: ceftazidime

KEY WORDS
serum

REFERENCE
Deeter,R.G.; Weinstein,M.P.; Swanson,K.A.; Gross,J.S.; Bailey,L.C. Crossover assessment of serum bactericidal activity and pharmacokinetics of five broad-spectrum cephalosporins in the elderly, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1007–1013.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL 8.5% phosphoric acid. Condition an NH2 SPE cartridge with 1 mL hexane. 500 μL Plasma + 25 μL 8.5% phosphoric acid + 250 μL 1 mg/mL coumarin-3-carboxylic acid in water, add to the C18 SPE cartridge, wash with 500 μL water, wash with 1 mL 8.5% phosphoric acid, wash with 5% MeOH:8.5% phosphoric acid 20:1, elute with 1 mL MeOH: 8.5% phosphoric acid 60:40 into the NH2 SPE cartridge. Wash the NH2 SPE cartridge with 1 mL hexane, wash with 1 mL MeCN, elute with 1 mL water:10% ammonium sulfate 95:5, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4.6 C18
Mobile phase: Water:2 mM tetramethylammonium hydroxide in MeOH:acetic acid 60:40: 0.5
Flow rate: 0.8
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 5
Internal standard: coumarin-3-carboxylic acid (13)

OTHER SUBSTANCES
Extracted: cefazolin, cefaclor, cephalexin

KEY WORDS
plasma; SPE

REFERENCE
Moore,C.M.; Sato,K.; Hattori,H.; Katsumata,Y. Improved HPLC method for the determination of cephalosporins in human plasma and a new solid-phase extraction procedure for cefazolin and ceftizoxime [letter], *Clin.Chim.Acta*, **1990**, *190*, 121–123.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 20 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 800 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 50-fold with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:20 mM KH_2PO_4 7:93 containing 10 mM triethylamine, adjusted to pH 4.8 with HCl
Flow rate: 1.5
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: ceftazidime, ceftriaxone, metronidazole
Noninterfering: degradation products

KEY WORDS
saline; injections

REFERENCE
Rivers,T.E.; Webster,A.A. Stability of ceftizoxime sodium, ceftriaxone sodium, and ceftazidime with metronidazole in ready-to-use metronidazole bags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2568–2570.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 μL onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 0.3 5 μm ODS C18 (Nomura); B 150 × 0.3 5 μm ODS C18 (Nomura)
Mobile phase: A 10 mM ammonium acetate adjusted to pH 5 with acetic acid; B MeOH:water:acetic acid 40:60:0.5
Flow rate: A 0.1; B 0.004
Injection volume: 100
Detector: UV 262

CHROMATOGRAM
Retention time: 5.75
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: cefaclor, cephaloridine, cefazolin

KEY WORDS
microbore; column-switching

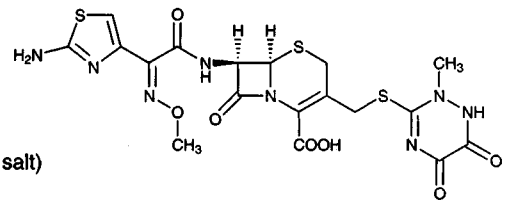
REFERENCE
Moore,C.M.; Sato,K.; Katsumata,Y. High-performance liquid chromatographic determination of cephalosporin antibiotics using 0.3 mm I.D. columns, *J.Chromatogr.*, **1991**, *539*, 215–220.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 5:95
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Ceftriaxone



Molecular formula: $C_{18}H_{18}N_8O_7S_3$
Molecular weight: 554.59
CAS Registry No.: 73384-59-5, 104376-79-6 (sodium salt)
Merck Index: 2001

SAMPLE

Matrix: blood
Sample preparation: After serum deproteination with MeCN, extract the serum samples with 1 mL dichloromethane. Inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:pH 7.0 phosphate buffer 25:75
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM

Internal standard: o-anisic acid
Limit of quantitation: 10 μg/mL

KEY WORDS

serum

REFERENCE

Belliveau,P.P.; Freeman,C.D.; Nicolau,D.P.; Nightingale,C.H.; Tessier,P.R.; Quintiliani,R. Serum bactericidal activity of ceftizoxime and ceftriaxone against pathogens associated with community-acquired and nosocomial pneumonias, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1024–1027.

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 10.8 (mobile phase A), 12.6 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum 1:10 with cold MeOH or filter (Millipore Ultraspec-MC, molecular weight limit 10000), inject an aliquot.

HPLC VARIABLES
Column: 25 × 4.6 5 μm C18
Mobile phase: MeCN:1 M pH 7 phosphate buffer:water 50:1:49 containing 3 g/L hexadecyltrimethylammonium bromide
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 6-7
Limit of quantitation: 500 ng/mL (filtered sample)

KEY WORDS
serum; ultrafiltrate; pharmacokinetics

REFERENCE
Hayward,C.J.; Nafziger,A.N.; Kohlhepp,S.J.; Bertino,J.S.,Jr. Investigation of bioequivalence and tolerability of intramuscular ceftriaxone injections using 1% lidocaine, buffered lidocaine, and sterile water diluents, *Antimicrob.Agents Chemother.*, **1996**, *40*, 485–487.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 245.2

CHROMATOGRAM
Retention time: 5.34

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute ceftriaxone powder with water to a drug concentration of 100 mg/mL. Dilute 10 μL 100 mg/mL ceftriaxone in water and 10 μL 100 mg/mL ceftazidime in water to 5 mL with water, inject a 10 μL aliquot of the solution.

HPLC VARIABLES
Column: 100 × 4.6 4 μm Novapak C18
Mobile phase: MeCN:7.5 mM pH 7.0 phosphate buffer 40:60 containing 2 g/L tetrabutylammonium phosphate
Flow rate: 1.5
Injection volume: 10
Detector: UV 242

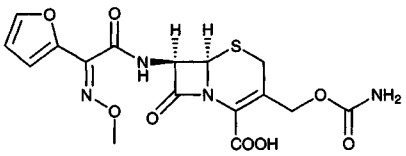
CHROMATOGRAM
Retention time: 2.3
Internal standard: ceftazidime (1.6)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Plumridge,R.J.; Rieck,A.M.; Annus,T.P.; Langton,S.R. Stability of ceftriaxone sodium in polypropylene syringes at -20, 4, and 20°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2320–2323.

Cefuroxime



Molecular formula: $C_{16}H_{16}N_4O_8S$
Molecular weight: 424.39
CAS Registry No.: 55268-75-2, 56238-63-2 (sodium salt), 64544-07-6 (axetil), 100680-33-9 (pivoxetil)
Merck Index: 2002
Lednicer No.: 3 216

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 5.3 (mobile phase A), 6.4 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 278.3

CHROMATOGRAM
Retention time: 16.293 (peak 1), 16.552 (peak 2)

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bronchoalveolar lavage fluid
Sample preparation: Condition a C18 SPE cartridge with 2 mL MeCN, 1 mL water, and 1 mL 50 mM pH 6.0 citrate buffer. Centrifuge bronchoalveolar lavage fluid at 1150 g and 4° for 10 min, dilute 200-400 μL of the supernatant with 1 mL 50 mM pH 6.0 citrate buffer and add 30 μL 1 mg/mL cephaloridine in 20 mM pH 6.0 phosphate buffer. Add to the SPE cartridge, wash with 1 mL 50 mM pH 6.0 citrate buffer, 500 μL water, two 400 μL portions of MTBE, and three 1 mL portions of MeCN. Dry the cartridge between each washing under full vacuum for at least 1 min. Elute with 1 mL MeOH, evaporate the eluate under nitrogen at 40°, dissolve the residue in 55 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 5 μm Symmetry C18 Sentry (Waters)
Column: 150 × 3.9 5 μm 100 Å Symmetry C18 (Waters)
Mobile phase: MeCN:50 mM pH 3.2 ammonium phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 9.17
Internal standard: cephaloridine (6.25)
Limit of detection: 1 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
SPE

REFERENCE
Rosseel,M.T.; Peleman,R.; Van Hoorbeke,H.; Pauwels,R.A. Measurement of cefuroxime in human bronchoalveolar lavage fluid by high-performance liquid chromatography after solid-phase extraction, *J.Chromatogr.B*, **1997**, *689*, 438–441.

SAMPLE
Matrix: milk

Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 19.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 18:82 (Buffer was 20 mM phosphoric acid containing 10 mM sodium decanesulfonate.)
Flow rate: 1
Injection volume: 200
Detector: UV 260

REFERENCE

Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Retention time: 2.2
Limit of detection: 60 nM

OTHER SUBSTANCES

Simultaneous: cefoperazone, cefoxitin, ceftazidime, cephalexin, cephradine

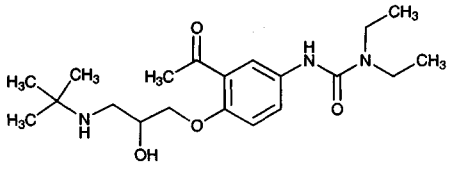
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Celiprolol



Molecular formula: $C_{20}H_{33}N_3O_4$
Molecular weight: 379.50
CAS Registry No.: 56980-93-9, 57470-78-7 (HCl)
Merck Index: 2007
Lednicer No.: 4 27

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 µL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 µL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 µm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 µm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4 K_3PO_4 buffer 27:73
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 242, UV 230

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Extracted: metoprolol, tiracizine, talinolol, oxprenolol, metabolites

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE

Matrix: blood
Sample preparation: Add 50 µL 1.4 µg/mL acebutolol, 1 mL 67 mM pH 7.4 phosphate buffer, 200 µL 1 M NaOH, and 6 mL MTBE to 1 mL plasma. Shake for 10 min, centrifuge at 1300 g at 4° for 5 min, freeze the aqueous layer in acetone/dry ice. Add 200 µL 10 mM HCl to the organic layer, shake for 10 min, centrifuge at 1300 g for 5 min. Freeze the aqueous layer, discard the organic phase, eliminate traces of the organic layer using a stream of cold air over the aqueous layer for 3-4 min. Thaw the aqueous layer, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb hexyl
Mobile phase: MeCN:15 mM pH 3.5 KH_2PO_4 containing 0.05% triethylamine 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 238

CHROMATOGRAM
Retention time: 4.2
Internal standard: acebutolol (3.3)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma

REFERENCE
Verbesselt,R.; Zugravu,A.; Tjandramaga,T.B.; De Schepper,P.J. Liquid chromatographic determination of total celiprolol or (S)-celiprolol and (R)-celiprolol simultaneously in human plasma, *J.Chromatogr.B*, **1996**, *683*, 231–236.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 1.4 μg/mL acebutolol, 1 mL 67 mM pH 7.4 phosphate buffer, 200 μL 1 M NaOH, and 6 mL MTBE to 1 mL plasma. Shake for 10 min, centrifuge at 1300 g at 4° for 5 min, freeze the aqueous layer in acetone/dry ice. Evaporate the organic layer to dryness with a stream of air at 40°. Add 100 μL 0.005% R(-)-1-(1-naphtyl)ethyl isocyanate to the dry residue, vortex, let stand stoppered for 30 min, evaporate with a stream of air at 35°, redissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil BDS C18
Mobile phase: MeCN:15 mM pH 3.5 KH_2PO_4 buffer containing 0.05% triethylamine 50:50
Flow rate: 1
Injection volume: 20
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 16.6 (R), 18 (S)
Internal standard: acebutolol (11)
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Verbesselt,R.; Zugravu,A.; Tjandramaga,T.B.; De Schepper,P.J. Liquid chromatographic determination of total celiprolol or (S)-celiprolol and (R)-celiprolol simultaneously in human plasma, *J.Chromatogr.B*, **1996**, *683*, 231–236.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg RP 18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 50 ng propranolol + 1 mL pH 9 borate buffer, add to SPE cartridge, wash with 5 mL pH 3.15 phosphate buffer, wash with 3 mL water, wash with 500 μL MeCN:water:phosphate buffer 62:32:6, elute with 1 mL MeCN:water:phosphate buffer 62:32:6, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:water:phosphate buffer 62:32:6
Flow rate: 1
Injection volume: 100
Detector: F ex 335 em 480 (celiprolol) or ex 290 em 350 (propranolol)

CHROMATOGRAM
Retention time: 9
Internal standard: propranolol (12)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Rostock,G.; Günzel,R.; Glöckl,D. Solid-phase extraction and direct high-performance liquid chromatographic determination of celiprolol in plasma, *Int.J.Clin.Pharmacol.Ther.Toxicol.*, **1992**, *30*, 512–513.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cyanopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 250 μL MeOH:2-aminoheptane 99.8:0.2 at 1.5 mL/min, pass 750 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeCN:buffer 25:75 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 250
Detector: F ex 350 em 480

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL 20 μg/mL deacetyldiltiazem in water + 1 mL pH 10 borate buffer + 0.5 g NaCl, vortex for 30 s, add 6 mL hexane:isopropanol 95:5, vortex for 30 s, shake for 10 min, centrifuge at 700 g for 10 min. Remove the organic layer and add it to 200 μL 5 mM sulfuric acid, vortex for 30 s, shake for 10 min, centrifuge at 700 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:buffer 22.5:22.5:55 (Buffer was 7.5 g sodium acetate trihydrate + 0.6 g 1-heptanesulfonic acid in 1 L water, adjust pH to 4.5 with acetic acid.)
Flow rate: 1.5
Injection volume: 40
Detector: UV 237

CHROMATOGRAM

Retention time: 3.2
Internal standard: deacetyldiltiazem (7)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Simultaneous: verapamil, propranolol, diltiazem, desipramine
Noninterfering: atenolol, aspirin, caffeine, ibuprofen, lidocaine, metoprolol, nifedipine

KEY WORDS

plasma

REFERENCE

Rutledge,D.R.; Abadi,A.H.; Lopez,L.M. Simultaneous determination of verapamil and celiprolol in human plasma, *J.Chromatogr.Sci.*, **1994**, *32*, 153–156.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 233

CHROMATOGRAM

Retention time: 4.36
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ke-

tamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma or urine + 0.1 (plasma) or 1 (urine) mL 1 μg/mL (R)-(+)-propranolol in buffer + 5 mL dichloromethane:isopropanol 95:5, shake for 15 min, centrifuge at 10° at 2500 g for 20 min. Remove 4 mL of the organic layer, add 4 mL dichloromethane:isopropanol 95:5 to the aqueous layer, shake for 15 min, centrifuge at 10° at 2500 g for 20 min. Combine the organic layers and evaporate them to dryness in a vacuum centrifuge at 30°, reconstitute the residue in 250 μL mobile phase. (Buffer was 3.092 g boric acid, 3.728 g KCl, and 1.756 g NaOH in 1 L water, pH 10.0.)

HPLC VARIABLES

Guard column: 50 × 4.6 10 μm Chiralcel OD
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: n-Hexane:isopropanol:diethylamine 85:20:0.1
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 480 (celiprolol) or ex 295 em 345 (propranolol)

CHROMATOGRAM

Retention time: 12.7 (S)-(-), 17.0 (R)-(+)
Internal standard: (R)-(+)-propranolol (9.5)
Limit of detection: 2.5 ng/mL (urine), 1.5 ng/mL (plasma)

OTHER SUBSTANCES

Noninterfering: metabolites, acebutolol, amiloride, atenolol, captopril, carteolol, clonidine, dihydralazine, furosemide, hydrochlorothiazide, labetalol, α-methyldopa, metoprolol, nifedipine, oxprenolol, penbutolol, pindolol, reserpine, triamterene, verapamil

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Hartmann,C.; Krauss,D.; Spahn,H.; Mutschler,E. Simultaneous determination of (*R*)- and (*S*)-celiprolol in human plasma and urine: high-performance liquid chromatographic assay on a chiral stationary phase with fluorimetric detection, *J.Chromatogr.*, **1989**, *496*, 387–396.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 232.2

CHROMATOGRAM
Retention time: 11.493

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 30:70 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 2.86 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 5.25

OTHER SUBSTANCES
Also analyzed: acebutolol, bunitrolol, carazolol, esmolol, mepindolol, metoprolol, timolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions

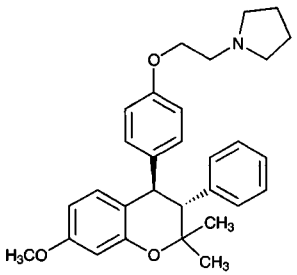
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.29

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Centchroman



Molecular formula: $C_{30}H_{35}NO_3$
Molecular weight: 457.61
CAS Registry No.: 78994-24-8, 51023-56-4 (HCl)
Merck Index: 2018

SAMPLE

Matrix: blood
Sample preparation: 1-2 mL Serum + 3 mL diethyl ether, vortex for 15 s, centrifuge at 1700 rpm for 5 min, freeze in liquid nitrogen, remove the organic layer. Extract twice more and combine the organic layers, evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 10 μm cyano guard column (Kontron)
Column: 110 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:10 mM KH_2PO_4:0.3 mM orthophosphoric acid 60:32:8
Flow rate: 1
Detector: F ex 279 em 305

CHROMATOGRAM

Retention time: 6.2
Limit of detection: 2 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Paliwal,J.K.; Gupta,R.C.; Grover,P.K.; Asthana,O.P.; Srivastava,J.S.; NityaNand,S. High performance liquid chromatographic (HPLC) determination of centchroman in human serum and application to single-dose pharmacokinetics, *Pharm.Res.*, **1989**, *6*, 1048–1051.

SAMPLE

Matrix: blood, milk
Sample preparation: Serum. 500 μL Serum + 50 μL 1% KOH, vortex for 15 s, add 3 mL diethyl ether, vortex for 1 min, centrifuge at 100 g for 10 min, freeze in liquid nitrogen, remove the organic layer, repeat the extraction of the aqueous layer. Combine the organic layers and evaporate them to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an 50 μL aliquot. Milk. 500 μL Milk + 2 mL MeCN, vortex for 1 min, let stand at 4° for 30 min, centrifuge at 1000 g for 10 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL 100 mM pH 8.7 K_2HPO_4, add 3 mL diethyl ether, vortex for 1 min, centrifuge at 100 g for 10 min, freeze in liquid nitrogen, remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an 50 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spheri-5 cyano
Column: 100 × 4.6 5 μm Spheri-5 cyano
Mobile phase: MeCN:buffer 58:42 (Buffer was 20 mM KH_2PO_4 adjusted to pH 3 with 20% orthophosphoric acid.)
Flow rate: 1.5

Injection volume: 50
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 5.6
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Lal,J.; Paliwal,J.K.; Grover,P.K.; Gupta,R.C. Simultaneous liquid chromatographic determination of centchroman and its 7-demethylated metabolite in serum and milk, *J.Chromatogr.B*, **1994**, *658*, 193–197.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder 20 tablets, weigh out amount equivalent to 10 mg centchroman hydrochloride, extract three times with 5 mL portions of MeOH, combine the extracts, centrifuge, make up to 25 mL with MeOH, remove a 250 μL aliquot and make it up to 25 mL with mobile phase.

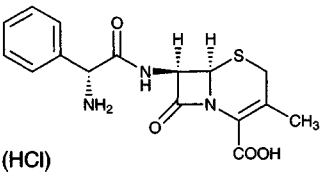
HPLC VARIABLES
Column: 250 × 4.6 5 μm Phenomenex ODS
Mobile phase: MeCN:water:10% tetramethylammonium hydroxide in water 80:20:0.4, adjusted to pH 7.6 with 100 mM orthophosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 24.0 (trans), 34.0 (cis)
Limit of detection: 100 ng (trans), 50 ng (cis)
Limit of quantitation: 180 ng (trans), 110 ng (cis)

KEY WORDS
tablets; bulk

REFERENCE
Dwivedi,A.K.; Sirkar,K.P.; Bhatt,G.R.; Seth,R.K.; Singh,S.; Sarin,J.P.S. Determination of *cis*- and *trans*-centchroman in its dosage forms by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *663*, 187–190.

Cephalexin



Molecular formula: $C_{16}H_{17}N_3O_4S$
Molecular weight: 347.39
CAS Registry No.: 15686-71-2, 23325-78-2 (monohydrate), 105879-42-3 (HCl)
Merck Index: 2021
Lednicer No.: 1 417; 2 439; 4 182

SAMPLE

Matrix: blood
Sample preparation: Mix 10 mL serum with an equal volume MeCN, vortex for 20 s, centrifuge at 2040 g for 10 min. Dilute the supernatant to 50 mL with water. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm Nova-Pak C18 Radial-Pak
Mobile phase: Gradient. A was MeCN:MeOH:10 mM pH 4.7 acetate buffer 2:11:87. B was MeCN:MeOH:10 mM pH 4.7 acetate buffer 11:2:87. A:B 100:0 for 5 min, to 70:30 over 10 min, to 0:100 over 12 min
Flow rate: 1
Injection volume: 50
Detector: E, pulsed electrochemical detector with Ag/AgCl reference electrode, stainless steel counter electrode and circular gold working electrode using indirect pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD)

CHROMATOGRAM

Retention time: 4
Limit of detection: 30 ppb (indirect PAD), 10 ppb (integrated PAD)

OTHER SUBSTANCES

Simultaneous: cefaclor, cefadroxil, cefazolin, cefotaxime, cefoxitin, cefsulodin, cefuroxime, cephaloglycin, cephalothin, cephradine

KEY WORDS

pig; serum

REFERENCE

Yun,E.K.; Prince,A.J.; McMillin,J.E.; Welch,L.E. High-performance liquid chromatographic separation and electrochemical detection of cephalosporins, *J.Chromatogr.B*, **1998**, *712*, 145–152.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL water + 50 μL 25 μg/mL cefprozil + 100 μL 1 M hydrochloric acid, vortex briefly. Filter (Centrifree micropartition unit) 1 mL mixture while centrifuging at 2000 g for 10 min. Inject a 25 μL aliquot of the ultrafiltrate. Urine. 250 μL urine + 500 μL 250 μg/mL cefprozil + 4.25 mL 10 mM pH 3.5 acetate buffer, vortex briefly. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleogil C18 (plasma) or 125 × 4.6 5 μm Lichrosorb C18 (urine)
Mobile phase: MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 8:92 (plasma) or MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 10:90 (urine)
Flow rate: 1.2 (plasma), 1.0 (urine)
Injection volume: 25
Detector: UV 260

CHROMATOGRAM
Retention time: 12 (plasma), 9.7 (urine)
Internal standard: cefprozil (9.2) (plasma), (6.3) (urine)
Limit of quantitation: 200 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Barbhaiya,R.H. A pharmacokinetic comparison of cefadroxil and cephalexin after administration of 250, 500 and 1000 mg solution doses, *Biopharm.Drug Dispos.*, **1996**, *17*, 319–330.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.875 (peak 1), 4.792 (peak 2)

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: milk, tissue
Sample preparation: Milk. Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 18.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. Tissue. Blend 5

g tissue, 5 mL water, 2 mL 100 mM tetraethylammonium chloride (for liver and kidney 1 mL 200 mM tetraethylammonium chloride and 1 mL 5 mM KH_2PO_4), and 40 mL MeCN at half power for 1 min, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate (20 mL for liver and kidney), add 2 mL buffer, add 5 mL water, add 5 mL t-butanol, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Proceed as above. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer A 35:65 (milk) or MeCN:buffer B 33:67 (tissue) (Buffer A was 10 mM phosphoric acid containing 5 mM potassium dihydrogen phosphate and 5 mM sodium dodecyl sulfate. Buffer B was 6.7 mM phosphoric acid containing 3.3 mM potassium dihydrogen phosphate and 2.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 260

KEY WORDS
muscle; liver; kidney

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:30 mM pH 7.0 sodium phosphate buffer 15:85
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 4
Limit of detection: 60 nM

OTHER SUBSTANCES
Simultaneous: cefoperazone, cefoxitin, cefuroxime, ceftazidime, cephradine

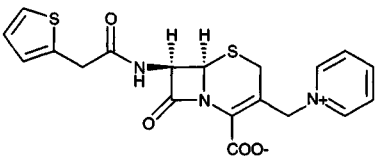
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Cephaloridine



Molecular formula: $C_{19}H_{17}N_3O_4S_2$
Molecular weight: 415.49
CAS Registry No.: 50-59-9
Merck Index: 2025
Lednicer No.: 1 417

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 252

CHROMATOGRAM

Retention time: 4.0 (mobile phase A), 4.7 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 11.52:0.48:88, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: cefoperazone (7.5)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: cefotaxime
Interfering: cefoxitin, cephalexin

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL plasma + 300 μL 5 μg/mL cefotaxime in pH 3.5 10 mM acetate buffer and keep at 4°. Inject 100 μL onto column A with mobile phase A. After 5 min backflush column A with mobile phase B onto column B for 3 min. Re-equilibrate column A with mobile phase A for 16 min.

HPLC VARIABLES
Column: A 40 × 2 37-50 μm Corasil RP C18; B 20 × 4 25-40 μm Lichrosorb RP-8 + 250 × 4 Partisil ODS-3
Mobile phase: A 10 mM pH 3.5 acetate buffer; B MeCN:20 mM pH 4.3 acetate buffer 15:85
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 10.3
Internal standard: cefotaxime
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: cephalexin, cefoxitin, cefuroxime
Noninterfering: cefotiam, cefadroxil, cefazolin, cefoperazone, cephalothin, cefamandole, aspirin, diclofenac, alclofenac, lonazolac, piroxicam, ibuprofen, indomethacin, ketoprofen, naproxen, phenylbutazone, mefenamic acid, caffeine

KEY WORDS
plasma; column-switching; rat; human

REFERENCE
Lee,Y.J.; Lee,H.S. Simultaneous determination of cefoxitin, cefuroxime, cephalexin and cephaloridine in plasma using HPLC and a column-switching technique, *Chromatographia*, **1990**, *30*, 80–84.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 9 (broad peak)
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 20 × 3.9 5 μm Symmetry C18 Sentry (Waters)
Column: 150 × 3.9 5 μm 100 Å Symmetry C18 (Waters)
Mobile phase: MeCN:50 mM pH 3.2 ammonium phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 40
Detector: UV 280

CHROMATOGRAM

Retention time: 6.25

OTHER SUBSTANCES

Simultaneous: cefuroxime

REFERENCE

Rosseel,M.T.; Peleman,R.; Van Hoorbeke,H.; Pauwels,R.A. Measurement of cefuroxime in human bronchoalveolar lavage fluid by high-performance liquid chromatography after solid-phase extraction, *J.Chromatogr.B*, **1997**, *689*, 438–441.

SAMPLE

Matrix: solutions

Sample preparation: Inject 100 μL onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 × 0.3 5 μm ODS C18 (Nomura); B 150 × 0.3 5 μm ODS C18 (Nomura)
Mobile phase: A 10 mM ammonium acetate adjusted to pH 5 with acetic acid; B MeOH: water:acetic acid 40:60:0.5
Flow rate: A 0.1; B 0.004
Injection volume: 100
Detector: UV 262

CHROMATOGRAM

Retention time: 7.03
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: cefaclor, cefazolin, ceftizoxime

KEY WORDS

microbore; column-switching

REFERENCE

Moore,C.M.; Sato,K.; Katsumata,Y. High-performance liquid chromatographic determination of cephalosporin antibiotics using 0.3 mm I.D. columns, *J.Chromatogr.*, **1991**, *539*, 215–220.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 Spheri 5 ODS-224
Mobile phase: 100 mM sodium dodecyl sulfate, pH 6.72
Flow rate: 1
Injection volume: 10
Detector: UV 260

CHROMATOGRAM

Retention time: 6.5

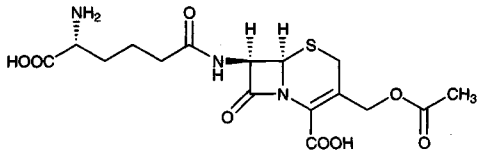
OTHER SUBSTANCES

Simultaneous: cefazolin, cephalothin, cephalexin, cephradine, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE

Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

Cephalosporin C



Molecular formula: $C_{16}H_{21}N_3O_8S$
Molecular weight: 415.42
CAS Registry No.: 61-24-5
Merck Index: 2026

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 31

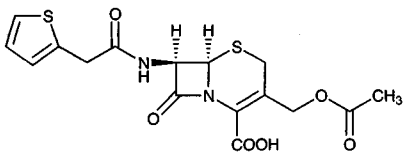
OTHER SUBSTANCES

Simultaneous: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefazolin, cefoperazone, cefotaxime, ceftazidime

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

Cephalothin



Molecular formula: $C_{16}H_{16}N_2O_6S_2$
Molecular weight: 396.44
CAS Registry No.: 153-61-7, 58-71-9 (sodium salt)
Merck Index: 2028
Lednicer No.: 1 420

SAMPLE

Matrix: aqueous humor
Sample preparation: 150 μL Aqueous humor + 50 μL 400 mM HCl, mix, add 700 μL chloroform:1-pentanol 3:1, mix by swirl-mixing for 5 min, centrifuge at 300 g for 5 min, discard the organic layer. Centrifuge the aqueous layer briefly, hold it at 4°, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Ultrasphere RP-ODS
Mobile phase: MeOH:water:buffer 40:48:12, the final pH was adjusted to 6.7 with triethylamine (Buffer was 50 mM pH 6.7 morpholinopropanesulfonic acid (MOPS)-triethylamine.)
Column temperature: 32
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.74
Internal standard: cephalothin

OTHER SUBSTANCES

Simultaneous: piperacillin, carbenicillin, mezlocillin, azlocillin, cefamandole, cefoxitin, cefuroxime, scopolamine, sulfamethoxazole, theophylline, ticarcillin, timolol
Noninterfering: acetazolamide, amitriptyline, atropine, carbachol, cefazolin, cefoperazone, cefotaxime, chlorpheniramine, codeine, diazepam, echothiophate, epinephryl borate, imipramine, prednisolone acetate, tropicamide, xylazine
Interfering: acetaminophen, ampicillin, caffeine, salicylic acid

KEY WORDS

rabbit; cephalothin is IS

REFERENCE

Riegel,M.A.; Ellis,P.P. High-performance liquid chromatographic assay for piperacillin in aqueous humor of the eye, *J.Chromatogr.*, **1988**, *424*, 177–181.

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate

Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 13.0
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cephaloridine, cefuroxime
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 19.2:0.8:20:80, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: cefamandole (4)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Place 500 μL serum in an ultrafree MC filter unit with a 10000 MW cutoff (Millipore), centrifuge at 16000 g for 30 min, inject 180 μL a aliquot of the filtrate. Tissue. Homogenize 100 mg tissue in 1 mL water using a Polytron homogenizer (Brinkman), centrifuge at 1000 g for 15 min, filter (Acrodisc CR PTFE 0.2 μm filter, prewet with water and MeOH) the supernatant, inject a 180 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak

Column: 100 × 8 10 μm μBondapak C18
Mobile phase: MeCN:5 mM KH_2PO_4:glacial acetic acid 22:77.5:0.5
Flow rate: 2
Injection volume: 180
Detector: UV 235

CHROMATOGRAM
Retention time: 13.55 (serum), 13.95 (tissue)
Internal standard: cephalothin

OTHER SUBSTANCES
Extracted: cefoxitin

KEY WORDS
cat; colon; serum; cephalothin is IS

REFERENCE
Cox,S.K.; Burnette,J.D.; Huss,B.T.; Frazier,D. Determination of cefoxitin in serum and tissue, *J.Chromatogr.B*, **1998**, *705*, 145–148.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Dilute plasma with three volumes of MeOH, centrifuge at 2000 rpm for 10 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot. Urine. Dilute urine with 0.1% phosphate buffer, centrifuge at 3000 rpm for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeOH:0.2% ammonium acetate 40:60
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; human; pharmacokinetics

REFERENCE
Sakata,Y. The pharmacokinetic studies of cephalothin, cefazolin and cefmetazole in the neonates and the premature babies, *Kurume Med.J.*, **1980**, *27*, 275–298.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 30 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 30
Limit of quantitation: 2370 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cefamandole, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: cell suspensions
Sample preparation: Add two volumes cold MeOH, centrifuge at 7080 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.5 μBondapak C18
Mobile phase: Gradient. MeOH:10 mM pH 6.8 K_2HPO_4 from 20:80 to 60:40 over 20 min
Flow rate: 1
Detector: UV 254

OTHER SUBSTANCES
Extracted: metabolites, cephaloglycin

KEY WORDS
rabbit; liver; kidney

REFERENCE
Williams,P.D.; Laska,D.A.; Tay,L.K.; Hottendorf,G.H. Comparative toxicities of cephalosporin antibiotics in a rabbit kidney cell line (LLC-RK_1), *Antimicrob.Agents Chemother.*, **1988**, *32*, 314–318.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeOH:water 30:70 containing 10 mM ammonium acetate
Flow rate: 2.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: cefazolin

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE

Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE

Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 28:72
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 7.2
Limit of detection: 500 ng/mL

REFERENCE

Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:90 mM perchloric acid 13.5:86.5. B was MeCN:300 mM perchloric acid 45:55. A:B from 100:0 to 0:100 over 7 min, maintain at 0:100.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: 7-aminocephalosproranic acid, cefadroxil, cefazolin, cefotaxime, cephalexin, cephaloridine, cephalosporin C, cephapirin, D-hydroxyphenylglycine

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:10 mM ammonium acetate 22:78
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Selective analysis of mutual displacement effects at the primary binding sites of phenoxymethylpenicillin and cephalothin bindings to human serum albumin, *J.Pharmacobiodyn.*, **1992**, *15*, 91–97.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 Spheri 5 ODS-224
Mobile phase: 100 mM sodium dodecyl sulfate, pH 3.00
Flow rate: 1
Injection volume: 10
Detector: UV 260

CHROMATOGRAM

Retention time: 4.5

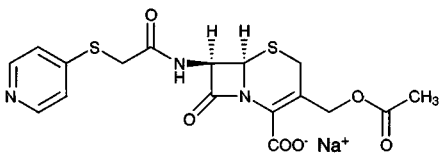
OTHER SUBSTANCES

Simultaneous: cefazolin, cephaloridine, cephalexin, cephradine, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE

Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

Cephapirin sodium



Molecular formula: $C_{17}H_{16}N_3NaO_6S_2$
Molecular weight: 445.5
CAS Registry No.: 24356-60-3, 21595-23-7 (cephapirin)
Merck Index: 2030
Lednicer No.: 2 441

SAMPLE

Matrix: blood
Sample preparation: Prepare an anion-exchange SPE cartridge in a 6 mL syringe barrel with a filter paper disc in the bottom. Pack with DEAE-A-25 Sephadex in PBS to a bed volume of 3 mL, wash with PBS, place filter paper on top. 500 μL Serum + 10 μL 1 mg/mL cefoxitin, add to SPE cartridge, add 500 μL PBS to SPE cartridge, wash with 4 mL PBS, elute with 5 mL 1 M NaCl, inject a 100 μL aliquot of the eluate. (PBS was 8 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KCl, and 0.2 g KH_2PO_4 in 1 L water, pH 7.2.)

HPLC VARIABLES

Column: 300 × 4 10 μm octadecylsilane
Mobile phase: MeCN:buffer 13:87 (Buffer was water adjusted to pH 2.8 with acetic acid, about 1.5 mL/min.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 270

CHROMATOGRAM

Retention time: 3.7
Internal standard: cefoxitin (7.8)
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, cefotaxime
Noninterfering: amikacin, amphotericin B, azathioprine, carbenicillin, chloral hydrate, cimetidine, dopamine, fluphenazine, furosemide, hydrochlorothiazide, insulin, levothyroxine, methylprednisolone, nitroglycerin, oxacillin, prednisone, procainamide, sulfamethoxazole, tolazamide, tolbutamide, triamterene, trimethoprim

KEY WORDS

serum; SPE

REFERENCE

Fasching,C.E.; Peterson,L.R. Anion-exchange extraction of cephapirin, cefotaxime, and cefoxitin from serum for liquid chromatography, *Antimicrob.Agents Chemother.*, **1982**, *21*, 628–633.

SAMPLE

Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3

Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 6.7
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefotaxime, cefoxitin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 18.24:0.76:81, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: cefoperazone (5.5)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood, milk
Sample preparation: 500 μL Milk or serum + 500 μL MeCN:MeOH:water 40:20:40, vortex for 10-15 s, centrifuge through an Amicon Centricon-10 filter (10000 Dalton cut-off) at 3000 g for 30 min, inject a 10-40 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 phenyl
Mobile phase: MeCN:MeOH:0.1% 85% phosphoric acid containing 5 mM sodium do decanesulfonate 20:5:75

Column temperature: 40
Flow rate: 0.4-0.5
Injection volume: 10-40
Detector: UV 291

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; cow; ultrafiltrate

REFERENCE
Tyczkowska,K.L.; Voyksner,R.D.; Aronson,A.L. Development of an analytical method for cephapirin and its metabolite in bovine milk and serum by liquid chromatography with UV-VIS detection and confirmation by thermospray mass spectometry, *J.Vet.Pharmacol.Ther.*, **1991**, *14*, 51–60.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in mobile phase at a concentration of 200 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: DMF:acetic acid:45% KOH (w/w):water 5:0.2:0.1:94.7
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13.3
Internal standard: acetanilide (18.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
MacNeil,L.; Rice,J.J.; Muhammad,N.; Lauback,R.G. Stability-indicating liquid chromatographic determination of cephapirin, desacetyl cephapirin and cephapirin lactone in sodium cephapirin bulk and injectable formulations, *J.Chromatogr.*, **1986**, *361*, 285–290.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 20 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: 980 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: milk
Sample preparation: Condition a Sep-Pak SPE cartridge with 3 mL MeOH and 5 mL water. 5 mL Milk + 10 mL 10 mM sodium acetate, mix, add to SPE cartridge, rinse in with 3 mL water, wash with 10 mL water, wash with 5 mL dichloromethane, dry with air for 1 min, add 500 μL mobile phase, elute with 1 mL MeCN:MeOH 75:25, evaporate eluate under reduced pressure until only a trace of liquid remains, make up to 500 μL with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 mm long Spheri-10 RP-18
Column: 150 × 4.6 5 μm Ultrasphere-ODS
Mobile phase: MeCN:MeOH:10 mM sodium acetate 112.5:37.5:850 (After cephapirin has eluted inject 500 μL MeCN:MeOH 75:25 to remove late-eluting compounds, allow 6 min for column to re-equilibrate. Wash column with 50 mL MeCN:MeOH 75:25 at end of each day.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.1
Limit of quantitation: 10 ppb

OTHER SUBSTANCES
Simultaneous: ampicillin, cloxacillin, penicillin G
Noninterfering: ceftiofur

KEY WORDS
cow; SPE

REFERENCE
MacIntosh,A.I. Liquid chromatographic determination of cephapirin residues in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 880–882.

SAMPLE
Matrix: milk
Sample preparation: 10 mL Milk + 2 mL 200 mM tetraethylammonium chloride, stir, slowly add 38 mL MeCN over 30 s, let stand for 5 min, decant the supernatant through a plug of glass wool. 40 mL Filtrate + 1 mL water, evaporate under reduced pressure to 1-2 mL, make up to 4 mL with water, filter (0.45 μm polyvinylidene difluoride). Inject 2 mL into an LC system (150 × 4.6 5 μm Supelcosil LC-18; MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 40:60 over 27 min, to 0:100 over 1 min; 1 mL/min; UV 210 and 295), collect

a 1.5 mL fraction at retention time for cephapirin (18 min), evaporate to 1 mL, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 15 mM phosphoric acid and 7.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 290

CHROMATOGRAM

Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES

Also analyzed: ampicillin, amoxicillin, penicillin G, ceftiofur, penicillin V, cloxacillin

KEY WORDS

cow

REFERENCE

Moats,W.A.; Harik-Khan,R. Liquid chromatographic determination of β-lactam antibiotics in milk: A multiresidue approach, *J.AOAC Int.*, **1995**, *78*, 49–54.

SAMPLE

Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM

Retention time: 8.78

OTHER SUBSTANCES

Extracted: ampicillin, ceftiofur, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS

SPE

REFERENCE

Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 19.8 min), evaporate to <1 mL under reduced pressure, add 200 μL 10 mM KH_2PO_4 containing 10 mM phosphoric acid and 10 mM sodium decanesulfonate, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 15 mM phosphoric acid containing 7.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 290

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid and 10 mM tetramethylammonium chloride from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100

g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: carbenicillin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:90 mM perchloric acid 13.5:86.5. B was MeCN:300 mM perchloric acid 45:55. A:B from 100:0 to 0:100 over 7 min, maintain at 0:100.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: 7-aminocephalosproranic acid, cefadroxil, cefazolin, cefotaxime, cephalexin, cephaloridine, cephalosporin C, cephalothin, D-hydroxyphenylglycine

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 20:80
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 260

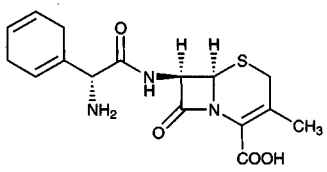
OTHER SUBSTANCES

Also analyzed: cefamandole

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cephradine



Molecular formula: $C_{16}H_{19}N_3O_4S$
Molecular weight: 349.41
CAS Registry No.: 38821-53-3, 58456-86-3 (dihydrate), 31828-50-9 (non-stoichiometric hydrate)
Merck Index: 2032
Lednicer No.: 2 440

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 4 mL water + 3 mL 10% trichloroacetic acid, centrifuge at 800-1000 g for 5 min. Remove 3 mL of the supernatant and add it to 3 mL buffer, add 1 mL 0.6% hydrogen peroxide in buffer, heat in a boiling water bath for 55 min, cool to room temperature, add 1 mL prewarmed 1 M Na_2HPO_4, add 7 mL acetone: chloroform 40:60, shake vigorously for 5 min, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL MeOH containing IS, inject a 20 μL aliquot. (Prepare buffer by dissolving 21 g citric acid in 200 mL 1 M NaOH, make up to 1 L with water, adjust pH to 2.5 with 100 mM HCl.)

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:water 60:40
Column temperature: 55
Injection volume: 20
Detector: F ex 345 em 420

CHROMATOGRAM

Retention time: 6 (?)
Internal standard: methyl anthranylate (9 (?))
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Interfering: ampicillin, cephalexin

KEY WORDS

plasma; derivatization

REFERENCE

Miyazaki,K.; Ohtani,K.; Sunada,K.; Arita,T. Determination of ampicillin, amoxicillin, cephalexin, and cephradine in plasma by high-performance liquid chromatography using fluorometric detection, *J.Chromatogr.*, **1983**, *276*, 478–482.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 200 μg/mL cefadroxil in water + 100 μL 6% trichloroacetic acid, vortex, centrifuge at 9000 g for 10 min, inject 25 μL supernatant.

HPLC VARIABLES

Guard column: Waters Guard-Pak C18
Column: 200 × 4.6 5 μm Nucleosil SA
Mobile phase: 20 mM Ammonium dihydrogen phosphate to final concentration of 20 mM in water:MeOH:MeCN 30:35:35. The pH was adjusted to 3.0 with concentrated phosphoric acid.

Flow rate: 1.5
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 10.5
Internal standard: cefadroxil
Limit of quantitation: 1000 ng/mL

KEY WORDS
serum

REFERENCE
Lindgren,K. Determination of cefadroxil in serum by high-performance liquid chromatography with cephradine as internal standard, *J.Chromatogr.*, **1987**, *413*, 347–350.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 100 μg/mL cephradine in water + 100 μL MeCN, vortex, centrifuge at 9000 g for 10 min. Remove 100 μL supernatant, evaporate to dryness at room temperature under reduced pressure, dissolve residue in 100 μL 20 mM NaH_2PO_4 adjusted to pH 3.5 with phosphoric acid, centrifuge at 9000 g for 5 min, inject 20 μL supernatant.

HPLC VARIABLES
Guard column: Waters Guard Pak C18
Column: 200 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 30:70, pH adjusted to 7.0 with NaOH (Buffer was 20 mM sodium phosphate and 5 mM tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5
Internal standard: cefaclor
Limit of detection: 1000 ng/mL

KEY WORDS
serum

REFERENCE
Lindgren,K. Determination of cefaclor and cephradine in serum by ion-pair reversed-phase chromatography, *J.Chromatogr.*, **1987**, *413*, 351–354.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 5 μg/mL cefixime in MeOH + 100 μL MeCN, vortex for 15 s, centrifuge at 14000 g for 2 min. Remove the supernatant and evaporate it under a stream of nitrogen, reconstitute in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Silica Guard Pak (Waters)
Column: 150 × 4.6 5μm Ultrasphere Octyl C8
Mobile phase: MeOH:12.5 mM pH 2.6 NaH_2PO_4 (pH adjusted with concentrated phosphoric acid) 20:80

Flow rate: 2
Injection volume: 50-80
Detector: UV 240

CHROMATOGRAM
Retention time: 21
Internal standard: cefixime (11)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: cefaclor, cefadroxil, cephalexin
Noninterfering: acetaminophen, cimetidine, diazepam, digoxin, ibuprofen, phenytoin, propranolol, salicylic acid, warfarin.

KEY WORDS
serum

REFERENCE
McAteer,J.A.; Hiltke,M.F.; Silber,B.M.; Faulkner,R.D. Liquid-chromatographic determination of five orally active cephalosporins -cefixime, cefaclor, cefadroxil, cephalexin, and cephradine -in human serum, *Clin.Chem.*, **1987**, *33*, 1788–1790.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma + 1 mL 6% trichloroacetic acid, mix, centrifuge at 4000 rpm for 10 min, inject an aliquot of the supernatant. Inject an aliquot of urine directly.

HPLC VARIABLES
Guard column: 10 × 4 7 μm Lichrosorb RP 18
Column: 250 × 4 7 μm Lichrosorb RP 18
Mobile phase: MeCN:25 mM pH 7 phosphate buffer 10:90
Flow rate: 1
Injection volume: 10
Detector: F ex 385 em 485 following post-column reaction. The column effluent mixed with 200 μg/mL fluorescamine in MeCN pumped at 0.25 mL/min and the mixture flowed through a 4.5 m × 0.25 mm ID coil of PTFE tubing to the detector; UV 260

CHROMATOGRAM
Limit of detection: 1.8 ng/mL (F), 2.7 ng/mL (UV)

OTHER SUBSTANCES
Also analyzed: cefaclor, cefroxadine, cephalexin
Noninterfering: amidopyrin, aspirin, barbital, caffeine, cefmenoxime, cefotaxime, ceftizoxime, ceftriaxone, cetazidime, diazepam, dibekacin, gentamycin, kanamycin, lidocaine, netilmicin, tetracaine, theophylline, tobramycin

KEY WORDS
post-column reaction; plasma; F detection may be less susceptible to interferences

REFERENCE
Blanchin,M.D.; Fabre,H.; Mandrou,B. Fluorescamine post-column derivatization for the HPLC determination of cephalosporins in plasma and urine, *J.Liq.Chromatogr.*, **1988**, *11*, 2993–3010.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 40 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 1800 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: solution

HPLC VARIABLES
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: MeOH:10 mM pH 6 acetate buffer 20:80
Column temperature: 40
Flow rate: 1
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: antipyrine, benzoic acid, mannitol, 3-O-methyl-D-glucose, L-lactic acid, phenylalanine

REFERENCE
Ogihara,T.; Tamai,I.; Tsuji,A. Application of fractal kinetics for carrier-mediated transport of drugs across intestinal epithelial membrane, *Pharm.Res.*, **1998**, *15*, 620–625.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeOH:10 mM phosphate buffer 27:73, pH 3.6
Column temperature: 27
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: ampicillin, cephalexin, cefaclor, amoxicillin

REFERENCE
Huang,H.-S.; Wu,J.-R.; Chen,M.-L. Reversed-phase high-performance liquid chromatography of amphoteric β-lactam antibiotics: effects of columns, ion-pairing reagents and mobile phase pH on their retention times, *J.Chromatogr.*, **1991**, *564*, 195–203.

SAMPLE
Matrix: solutions
Sample preparation: Adjust pH to 3.0 with HCl, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:20 mM pH 5.0 NaH_2PO_4 13:87
Flow rate: 1.6
Detector: UV 262

KEY WORDS
buffers

REFERENCE
Yuasa,H.; Amidon,G.L.; Fleisher,D. Peptide carrier-mediated transport in intestinal brush border membrane vesicles of rats and rabbits: cephradine uptake and inhibition, *Pharm.Res.*, **1993**, *10*, 400–404.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 Spheri 5 ODS-224
Mobile phase: 100 mM sodium dodecyl sulfate, pH 6.72
Flow rate: 1
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: cefazolin, cephalothin, cephaloridine, cephalexin, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE
Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 6
Limit of detection: 60 nM

OTHER SUBSTANCES
Simultaneous: cefoperazone, cefoxitin, cefuroxime, ceftazidime, cephalexin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

SAMPLE
Matrix: tissue
Sample preparation: Muscle, fat. 10 g Minced tissue + 1 mL 10 mM pH 3.0 phosphate buffer, let stand for 30 min, add 19 mL 5% trichloroacetic acid, chill to 5°, homogenize (Virtis model 45), centrifuge at 1000 g for 5 min, filter (0.2 μm), inject 2 mL of the filtrate onto column A with mobile phase A, elute to waste with mobile phase A for 1.5 min then flush contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent. Liver, kidney. 10 g Minced tissue + 1 mL 10 mM pH 3.0 phosphate buffer, let stand for 30 min, add 19 mL 5% trichloroacetic acid, chill to 5°, homogenize (Virtis model 45), centrifuge at 1000 g for 5 min, filter (0.2 μm). 10 mL Filtrate + 20 mL dichloromethane:isopropanol 95:5, stir for 2 min, centrifuge at 1000 g for 5 min. Discard the organic layer, add 250 μL concentrated ammonia solution to the aqueous layer, add 20 mL dichloromethane:isopropanol 95:5, stir for 2 min, centrifuge at 1000 g for 5 min. Discard the organic layer, restore the initial pH of the aqueous layer with concentrated HCl. Inject 2 mL of this solution onto column A with mobile phase A, elute to waste with mobile phase A for 1.5 min then flush contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES
Column: A 25 × 4 25-40 μm LiChroprep RP 18; B 4 × 4 5 μm LiChrospher 100 CH-18 +250 × 4 5 μm LiChrospher 100 CH-18
Mobile phase: A MeOH:10 mM pH 3.0 phosphate buffer 15:85; B MeOH:10 mM pH 3.0 phosphate buffer 30:70 (Every 30 injections change column A and column B guard column, flush with 60 mL MeOH:water 30:70 and 30 mL MeOH.)
Flow rate: 1
Injection volume: 2000
Detector: UV 260

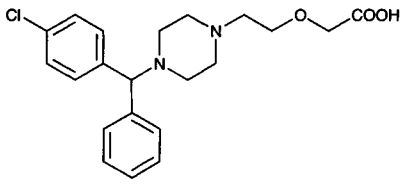
CHROMATOGRAM
Retention time: 15
Internal standard: cephradine

OTHER SUBSTANCES
Extracted: cephalexin

KEY WORDS
cow; muscle; fat; liver; kidney; cephradine is IS

REFERENCE
Leroy,P.; Decolin,D.; Nicolas,S.; Archimbault,P.; Nicolas,A. Residue determination of two coadministered antibacterial agents -cephalexin and colistin -in calf tissues using high-performance liquid chromatography and microbiological methods, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1837–1846.

Cetirizine



Molecular formula: $C_{21}H_{25}ClN_2O_3$
Molecular weight: 388.89
CAS Registry No.: 83881-51-0, 83881-52-1 (di HCl)
Merck Index: 2063
Lednicer No.: 4 118

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 3 μg/mL IS + 1 mL 1 M pH 5.0 sodium citrate buffer + 3 mL ethyl acetate, vortex 1 min, centrifuge at 4000 rpm for 15 min, remove organic layer, repeat extraction. Combine organic layers and add 200 μL 1.7% phosphoric acid, vortex 1 min, centrifuge 5 min, remove and discard ethyl acetate layer, remove traces of ethyl acetate from aqueous layer using a stream of nitrogen, inject.

HPLC VARIABLES

Column: radial 4 μm NovoPak C18 radial compression
Mobile phase: MeCN:buffer 46:54 (Buffer was 10 mM pH 2.9 KH_2PO_4 + 20 mM sodium 1-decanesulfonate.)
Flow rate: 1.4
Detector: UV 229

CHROMATOGRAM

Retention time: 5.0
Internal standard: P-265, an ethoxy derivative of cetirizine (6.8)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: hydroxyzine

KEY WORDS

serum

REFERENCE

Simons,K.J.; Watson,W.T.A.; Chen,X.Y.; Simons,F.E.R. Pharmacokinetic and pharmacodynamic studies of the H_1-receptor antagonist hydroxyzine in the elderly, *Clin.Pharmacol.Ther.*, **1989**, *45*, 9–14.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 100 μL buffer + 100 μL 25% perchloric acid, vortex for 10 s, centrifuge at 2000 g for 3 min, inject a 200 μL aliquot. (Buffer was 50 mM $(NH_4)H_4PO_4$ containing 0.5 mL/L triethylamine, adjust pH to 2.5 with 85% phosphoric acid.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Hyperspheres ODS (Shandon)
Column: 250 × 4.6 Spherisorb S5 ODS2
Mobile phase: MeCN:MeOH:50 mM pH 2.5 ammonium phosphate 33:9:58
Column temperature: 35
Flow rate: 2
Injection volume: 200
Detector: UV 211

CHROMATOGRAM

Retention time: 6.4

Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Moncrieff,J. Determination of cetirizine in serum using reversed-phase high-performance liquid chromatography with ultraviolet spectrophotometric detection, *J.Chromatogr.*, **1992**, *583*, 128–130.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 230

CHROMATOGRAM
Retention time: 5.59

Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifox-

amine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 5.0 phosphate buffer and 100 μL water, add 8 mL ethyl acetate, extract. Evaporate the organic layer, mix the residue with 500 μL 1.7% phosphoric acid and inject 200 μL aliquot of the lower layer. Tissue. Homogenize the brain with 2-fold the weight of water. Dilute 1500 μL brain homogenate with 500 μL 1 M pH 5.0 phosphate buffer, add 9 mL ethyl acetate, extract. Evaporate the organic layer, mix the residue with 400 μL 1.7% phosphoric acid, inject a 200 μL aliquot of the lower layer.

HPLC VARIABLES

Column: 150 × 4.6 Intersil PH
Mobile phase: MeCN:0.018% TFA 25:75
Flow rate: 0.7
Injection volume: 200
Detector: UV 230

CHROMATOGRAM

Limit of quantitation: 10 ng/mL (plasma), 15 ng/mL (brain)

KEY WORDS

brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE

Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.683

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.89 (A), 5.29 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone,

methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 25 μL 100 μg/mL IS in water + 1 mL 1 M pH 5 citrate buffer + 6 mL chloroform, shake horizontally for 10 min, centrifuge at 3015 g at 4° for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

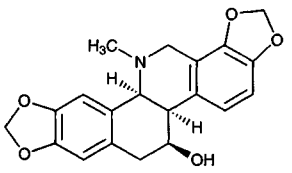
HPLC VARIABLES
Column: 250 × 4.6 Spherisorb 5ODS-2
Mobile phase: MeOH:THF:5 mM tetrabutylammonium phosphate (Pic A, Waters) 65:2:33
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 6.17
Internal standard: [2-[2-[4-(diphenylmethylene)-1-piperidinyl]ethoxy]ethoxy]-acetic acid (UCB J028) (5.53)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Rosseel,M.T.; Lefebvre,R.A. Determination of cetirizine in human urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 504–510.

Chelidonine



Molecular formula: $C_{20}H_{19}NO_5$
Molecular weight: 353.37
CAS Registry No.: 476-32-4, 20267-87-2 ((±)-form), 88200-01-5 ((-)-form)
Merck Index: 2095

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 3.625

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 25:1.5:0.5:73
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 6

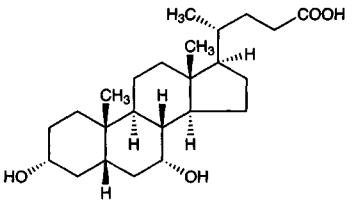
OTHER SUBSTANCES

Simultaneous: theobromine, theophylline, caffeine, 8-chlorotheophylline

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Chenodiol



Molecular formula: $C_{24}H_{40}O_4$
Molecular weight: 392.58
CAS Registry No.: 474-25-9
Merck Index: 2096

SAMPLE

Matrix: bile

Sample preparation: 200 μL Bile + 4 M NaOH:MeOH 50:50, heat at 80° for 16 h, adjust pH to 1.5 with 6 M HCl, extract three times with 10 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dry dichloromethane, add 1 mg p-aminophenol, add 150 μL triethylamine, add at least a 3-fold molar excess of 2-bromo-1-methylpyridinium iodide, heat at 60° for 30 min, cool, concentrate under a stream of nitrogen, add 1 mL 100 mM HCl, add 1 mL ethyl acetate, shake vigorously, centrifuge at 2500 rpm for 5 min, inject an aliquot of the supernatant. (Prepare 2-bromo-1-methylpyridinium iodide by analogy with the preparation of 2-chloro-1-methylpyridinium iodide. Add 15 g methyl iodide to 13.9 g 2-bromopyridine in 3 mL acetone at 0°, stir at room temperature for 3 days. Filter the precipitate and wash it with 50 mL dry ether, dry under reduced pressure to give 2-bromo-1-methylpyridinium iodide (Bull. Chem. Soc. Japan 1977, 50, 1863).)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C-18
Mobile phase: MeOH:water:perchloric acid 75:25:0.1 containing 50 mM sodium perchlorate
Column temperature: 25 ± 0.1
Flow rate: 0.9
Detector: E, 0.75 v, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.5
Limit of detection: 2 ng

OTHER SUBSTANCES

Extracted: cholic acid, deoxycholic acid, lithocholic acid

KEY WORDS

derivatization

REFERENCE

Ikenoya,S.; Hiroshima,O.; Ohmae,M.; Kawabe,K. Electrochemical detector for high performance liquid chromatography. IV. Analysis of fatty acids, bile acids and prostaglandins by derivatization to an electrochemically active form, *Chem.Pharm.Bull.(Tokyo)*, **1980**, *28*, 2941–2947.

SAMPLE

Matrix: bile

Sample preparation: Extract bile with 20 volumes EtOH, boil on a hot water bath, cool, let stand overnight, filter (Toyo Roshi 5A paper), filter (0.45 μm), add 200 μg/mL testosterone acetate in EtOH (final IS concentration 100 μg/mL), inject a 5-10 μL aliquot.

HPLC VARIABLES

Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:30 mM phosphate buffer 10:60:30, pH 3.40
Flow rate: 0.5

Injection volume: 5-10
Detector: UV 200

CHROMATOGRAM

Retention time: 25 (taurine conjugate), 30 (glycine conjugate)
Internal standard: testosterone acetate (39)
Limit of detection: 50 ng

OTHER SUBSTANCES

Extracted: ursodiol, conjugates, bile acids, deoxycholic acid

REFERENCE

Nakayama,F.; Nakagaki,M. Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 287–293.

SAMPLE

Matrix: bile, blood
Sample preparation: Serum. 100-200 μL Serum + 1 mL MeOH, mix, sonicate for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute with 1 mL 50 mM pH 7.0 phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot. Bile. Mix 10 μL bile with 10 mL 50 mM pH 7.0 phosphate buffer, add a 1 mL aliquot to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 μm Model RCM-100 Radial-Pak A (Waters)
Mobile phase: Gradient. MeCN:MeOH:water 100:50:40 for 30 min then 100:50:20 (step gradient).
Flow rate: 2
Injection volume: 8
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 44
Internal standard: lauric acid (56)
Limit of detection: 10 pmole
Limit of quantitation: 50 pmole

OTHER SUBSTANCES

Extracted: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; serum; SPE

REFERENCE

Kamada,S.; Maeda,M.; Tsuji,A. Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, *J.Chromatogr.*, **1983**, *272*, 29–41.

SAMPLE

Matrix: bile, blood, feces, gastric contents, tissue

Sample preparation: Condition a Sep-Pak C18 cartridge with 2 mL 720 mM MeOH in water and 6 mL 100 mM pH 7.0 potassium phosphate buffer. Serum. 200 μL Serum + 1 mL MeCN, mix, sonicate for 10 min, centrifuge at 17000 g for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Liver. Homogenize (glass homogenizer) liver in 1 mL 720 mM EtOH in water, add 2 mL 720 mM EtOH in water, heat at 75° for 15 min, centrifuge at 17000 g for 10 min, remove the supernatant, extract the residue twice more. Combine the supernatants and evaporate them to dryness at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Bile. Dilute 20 μL bile with 10 mL 100 mM pH 7.0 potassium phosphate buffer. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Gastric juice. Dilute 1 mL gastric juice with 9 mL 100 mM pH 7.0 potassium phosphate buffer, sonicate for 10 min. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Feces. Dilute 1 g feces with 9 mL MeOH, mix thoroughly, sonicate for 10 min, centrifuge at 17000 g for 10 min. Remove a 1 mL aliquot of the supernatant and evaporate it to dryness, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere I.P. C18

Mobile phase: Gradient. A was MeCN:MeOH:water 100:50:75. B was MeCN:MeOH 100:50. A:B 100:0 for 7 min, to 70:30 over 0.5 min, maintain at 70:30 for 5 min, to 50:50 over 0.5 min, maintain at 50:50 over 7 min, to 25:75 over 1 min, maintain at 25:75 for 7 min.

Column temperature: 35

Flow rate: 1.7

Injection volume: 100
Detector: F

CHROMATOGRAM
Retention time: 21.3
Internal standard: lauric acid (24.5)
Limit of detection: 0.5 pmole

OTHER SUBSTANCES
Extracted: cholic acid, deoxycholic acid, glycinechenodeoxycholic acid, glycinecholic acid, glycinedeoxycholic acid, glycinelithocholic acid, glycineursodeoxycholic acid, lithocholic acid, ursodiol (ursodeoxycholic acid)

KEY WORDS
derivatization; SPE; liver; serum

REFERENCE
Güldütuna,S.; You,T.; Kurts,W.; Leuschner,U. High performance liquid chromatographic determination of free and conjugated bile acids in serum, liver biopsies, bile, gastric juice and feces by fluorescence labeling, *Clin.Chim.Acta*, **1993**, *214*, 195–207.

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Urine. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-200 μL urine 1:4 with 100 mM NaOH, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Serum. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-500 μL serum with 3.5 mL 100 mM NaOH, heat at 64° for 30 min, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Bile. Dilute 1:500 to 1:1000 with mobile phase, filter (0.22 μm, inject an aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL C18
Mobile phase: MeOH:15 mM ammonium acetate 80:20, apparent pH 6.0 ± 0.1
Flow rate: 0.3
Detector: MS, electrospray, Fisons VG TRIO 2000 quadrupole (6% of the mobile phase was diverted to the MS detector) or evaporative light scattering detector (Varex)

CHROMATOGRAM
Retention time: 17.60
Limit of detection: 15 pg

OTHER SUBSTANCES
Extracted: deoxycholic acid, ursodiol, bile acids, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycoursodeoxycholic acid, lithocholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid

KEY WORDS
serum; SPE; hamster; human; LC-MS

REFERENCE
Roda,A.; Gioacchini,A.M.; Cerrè,C.; Baraldini,M. High-performance liquid chromatographic-electrospray mass spectrometric analysis of bile acids in biological fluids, *J.Chromatogr.B*, **1995**, *665*, 281–294.

SAMPLE
Matrix: bile, formulations

Sample preparation: Bile. Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. 50 μL Bile + 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge (J. Pharm. Biomed. Anal. 1990, 8, 235). Evaporate to dryness under a stream of nitrogen, reconstitute with 2 mL MeOH, sonicate at 40° for 3 min, filter (0.2 μm). Add a 500 μL aliquot of the filtrate to 50 μL 0.01% KOH in MeOH, evaporate to dryness, reconstitute with 200 μL MeOH:water 10:90, sonicate at 40° for 3 min, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. Formulations. Powder capsule contents, weigh out amount containing about 25 mg compound, add 100 mL MeOH (water for bile acid salts), stir for 10 min, filter, dilute the filtrate 10-fold with water (or MeOH:water 10:90 for bile acid salts). Evaporate 50 μL 0.01% KOH in MeOH in to a tube, add a 200 μL aliquot of the diluted filtrate, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil RP-18

Mobile phase: Gradient. For bile use MeCN:water 60:40 for 10 min, to 80:20 over 10 min, maintain at 80:20 for 25 min, return to initial conditions over 5 min. For formulations use isocratic MeCN:water 78:22.

Flow rate: 1

Injection volume: 50

Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 26.5 (gradient), 12 (isocratic)

Internal standard: 6-methoxynaphthacyl ester of valproic acid (23 (gradient), 10.5 (isocratic))

Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Extracted: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; capsules; SPE

REFERENCE

Cavrini,V.; Gatti,R.; Roda,A.; Cerrè,C.; Roveri,P. HPLC-fluorescence determination of bile acids in pharmaceuticals and bile after derivatization with 2-bromoacetyl-6-methoxynaphthalene, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 761–770.

SAMPLE

Matrix: bile, gastric contents

Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5

mL water, and 5 mL MeOH. Mix 50 μL bile or 500 μL gastric juice with 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH: 40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge. Evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL initial mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH:30 mM sodium acetate 65:35, adjusted to pH 4.3 with phosphoric acid. B was MeOH:70 mM sodium acetate 90:10, adjusted to pH 4.3 with phosphoric acid. A:B 85:15 for 10 min, to 10:90 over 25 min, maintain at 10:90 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Extracted: cholic acid, deoxycholic acid, lithocholic acid, ursodiol

KEY WORDS
SPE

REFERENCE
Scalia,S. Group separation of free and conjugated bile acids by pre-packed anion-exchange cartridges, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH, 10 mL water, and 2 mL 100 mM pH 8.0 Tris-HCl buffer. 5-7 mL Serum + 19 volumes 100 mM pH 8.0 Tris-HCl buffer, sonicate for 10 min, add to the SPE cartridge, wash with 15 mL water, elute with 6-7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, dissolve residue in water, filter (Millipore GS 0.22 μm), wash filter, evaporate filtrates to dryness, reconstitute in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:20 mM KH_2PO_4 65:35, adjust pH to 5.3
Flow rate: 1.4
Injection volume: 30
Detector: UV 210

CHROMATOGRAM
Retention time: 15 (taurine conjugate), 21 (glycine conjugate)
Limit of detection: 40 nM (glycine conjugate), 30 nM (taurine conjugate)

OTHER SUBSTANCES
Extracted: conjugates, ursodiol, deoxycholic acid, bile acids

KEY WORDS
serum; SPE

REFERENCE

Linnet,K. A high-pressure liquid chromatogaphic-enzymatic assay for glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acid in serum, *Scand.J.Clin.Lab.Invest.*, **1982**, *42*, 455–460.

SAMPLE

Matrix: blood

Sample preparation: Condition a BondElut SPE cartridge with 5 mL EtOH and 5 mL water. 100 μL Serum + 250 ng deoxycholic acid 12-propionate + 1 mL 500 mM pH 7.0 phosphate buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL 1.5% EtOH, elute with 2 mL 90% EtOH. Evaporate a 400 μL aliquot of the eluate, add 100 μL 2 mg/mL 1-anthroyl nitrile in MeCN, add 0.16% quinuclidine in MeCN, heat at 60° for 20 min, add 50 μL MeOH, evaporate under nitrogen. Dissolve the residue in 1 mL 90% EtOH, add to a 18 × 6 100 mg column of PHP-LH-20 Sephadex at 0.2 mL/min, wash with 1 mL 90% EtOH, elute with 5 mL 100 mM acetic acid in 90% EtOH (free bile acids), elute with 5 mL 200 mM formic acid in 90% EtOH (glycine-conjugated bile acids), elute with 5 mL 300 mM pH 6.3 acetic acid-potassium acetate in 90% EtOH (taurine-conjugated bile acids). Evaporate each fraction, dissolve the residue in 100-200 μL MeOH, inject a 5-10 μL aliquot. (Preparation of PHP-LH-20 Sephadex is as follows. Suspend 75.7 g Sephadex LH-20 in 200 mL dichloromethane using a glass stirring rod (not a magnetic stirrer) for 30 min, add 19 mL boron trifluoride ethyl etherate, after 15 min add 50 mL 35% epichlorohydrin in dichloromethane at 1-2 mL/min (Caution! Epichlorohydrin is a carcinogen!), stir for another 30 min, filter, wash with EtOH, dry chlorohydroxypropyl Sephadex LH-20 at 50° (J.Chromatogr. 1971, 59, 45). Stir 27.2 g chlorohydroxypropyl Sephadex LH-20 in 100.5 mL piperidine at room temperature for 30 min, add 5.74 g KOH in 302 mL MeOH, heat at 50-60° for 3 h with occasional shaking, filter, wash with EtOH:water 50:50, wash with 200 mM acetic acid in EtOH:water 70:30, wash with EtOH:water 90:10 until washings become neutral, store in EtOH:water 90:10 (Clin. Chim. Acta 1978 87 141). Prepare 1-anthroyl nitrile as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroyl nitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18
Mobile phase: MeOH:0.3% pH 6.0 potassium phosphate buffer 5:1
Flow rate: 1.8
Injection volume: 10
Detector: F ex 370 em 470

CHROMATOGRAM

Retention time: 13
Internal standard: deoxycholic acid 12-propionate (20)
Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: ursodiol, cholic acid deoxycholic acid, conjugates

KEY WORDS
serum; SPE; derivatization

REFERENCE
Goto,J.; Saito,M.; Chikai,T.; Goto,N.; Nambara,T. Studies on Steroids. CLXXXVII. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling, *J.Chromatogr.*, **1983**, *276*, 289–300.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL serum to a Waters C18 SPE cartridge, wash with two 4 mL portions of water, wash with two 2 mL portions of MeOH:water 10:90, wash with two 2 mL portions of MeOH:water 20:80, wash with two 2 mL portions of MeOH:water 30:70, wash with two 2 mL portions of MeOH:water 50:50, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 80°, reconstitute the residue in 50 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Lichrosorb RP 18
Mobile phase: MeOH:30 mM KH_2PO_4 76:24
Flow rate: 1.2
Injection volume: 20
Detector: UV 201

CHROMATOGRAM
Retention time: 16
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: ursodiol (ursodeoxycholic acid)

KEY WORDS
serum; SPE

REFERENCE
Baillet-Guffroy,A.; Baylocq,D.; Rabaron,A.; Pellerin,F. Nuclear magnetic resonance spectrometry and liquid chromatography of two bile acid epimers: ursodeoxycholic and chenodeoxycholic acid, *J.Pharm.Sci.*, **1984**, *73*, 847–849.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize 20 μL serum with a pretreatment column (Autoserumout, Sekisui), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Medipola Bile column (Sekisui)
Mobile phase: Gradient. A was MeCN:MeOH:30 mM ammonium acetate 20:20:60. B was MeCN:MeOH:30 mM ammonium acetate 30:30:40. A:B from 100:0 to 80:20 over 10 min, to 0:100 over 27 min, maintain at 0:100 for 30 min.
Flow rate: 1
Detector: F ex 340 em 460 following post-column reaction detection. The effluent from the column was mixed with reagent pumped at 1 mL/min, the mixture flowed through a 20 × 4 3α-HSD column (Sekisui) containing bound 3α-hydroxysteroid dehydrogenase to the detector. (The reagent was 1.36 g/L KH_2PO_4, 372 mg/L disodium EDTA, 140 mg/L βNAD, and 450 μL/L 2-mercaptoethanol in water adjusted to pH 7.8 with 5 M KOH.)

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Extracted: ursodiol, deoxycholic acid, bile acids

KEY WORDS
post-column reaction; immobilized enzyme reactor; serum

REFERENCE
Adachi,Y.; Nanno,T.; Itoh,T.; Kurumi,Y.; Yamazaki,K.; Sawada,Y.; Yamamoto,T. Determination of individual serum bile acids in chronic liver diseases: fasting levels and results of oral chenodeoxycholic acid tolerance test, *Gastroenterol.Jpn.*, **1988**, *23*, 401–407.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Dilute 100-200 μL serum with 4 mL 400 mM sodium bicarbonate, add to the SPE cartridge, wash with 20 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, add 400 μg sodium carbonate, add 50 μL 20 mg/mL 18-crown-6 in MeCN, heat at 40° for 1 h, make up to 500 μL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak ODS
Mobile phase: Gradient. A was MeCN:MeOH:water 15:13.8:71.2. B was MeCN. A:B from 100:0 to 37:63 over 47 min (Waters convex curve + 2), to 0:100 over 0.1 min (Waters curve +9), maintain at 0:100 for 7.9 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 1 for 47 min then 1.5
Injection volume: 10
Detector: F ex 320 em 385

CHROMATOGRAM
Retention time: 45.23
Limit of detection: 80 nM

OTHER SUBSTANCES
Extracted: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, ursodiol

KEY WORDS
derivatization; serum; SPE

REFERENCE
Wang,G.F.; Stacey,N.H.; Earl,J. Determination of individual bile acids in serum by high performance liquid chromatography, *Biomed.Chromatogr.*, **1990**, *4*, 136–140.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1-5 mg bile acid in 500 μL chloroform with enough MeOH to make a solution, add a solution of 1-naphthyldiazomethane in ether until the reddish-orange color persists, if the color disappears within 1 h add more reagent, add 1 drop acetic acid to decompose excess reagent, make up to 1 mL, inject a 5 μL aliquot. (Preparation of 1-naphthyldiazomethane is as follows. Stir 6.7 g 1-naphthaldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h (Caution! Hydrazine hydrate is a carcinogen!). Remove the solid by filtration and recrystallize it twice from EtOH to give 1-naphthaldehyde hydrazone as white crystals (mp 91-92°). Stir 3.1 g 1-naphthaldehyde hydrazone, 5 g anhydrous sodium sulfate, 50 mL ether, 1 mL EtOH saturated with KOH, and 10 g yellow mercuric oxide for 5 h, filter (sintered glass), con-

centrate the filtrate under reduced pressure to give 1-naphthyldiazomethane as red crystals (mp 40-41°) (Bull. Chem. Soc. Japan 1967, 40, 691).)

HPLC VARIABLES
Column: 300 mm long μPorasil
Mobile phase: Hexane:THF:MeOH 75:30:2
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 12
Limit of detection: 20-30 ng

OTHER SUBSTANCES
Simultaneous: deoxycholic acid, 3,7-dihydroxy-12-ketocholanic acid, lithocholic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Matthees,D.P.; Purdy,W.C. Naphthyldiazomethane as a derivatizing agent for the high-performance liquid chromatography detection of bile acids, *Anal.Chim.Acta*, **1979**, *109*, 161–164.

SAMPLE
Matrix: bulk
Sample preparation: Add 5 mL of a 39.2 mg/mL solution in dry MeCN to 120 mg 1-(2,5-dihydroxyphenyl)-2-bromoethane and 100 μL triethylamine, heat at 70° for 2 h, dilute with 20 mL water, extract 3 times with diethyl ether. Combine the extracts and wash them with saturated sodium bicarbonate and water, dry over anhydrous sodium sulfate, evaporate, reconstitute, inject a 5 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethane is as follows. Slowly add 2.5 g phenyltrimethylammonium tribromide to a solution of 2',5'-dihydroxyacetophenone in 20 mL dry THF, stir at room temperature overnight (check by TLC with cyclohexane:ethyl acetate 70:30). Remove the precipitate by filtration and dry under reduced pressure, chromatograph using cyclohexane:ethyl acetate 70:30 to give 1-(2,5-dihydroxyphenyl)-2-bromoethane.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm 5 μm Hypersyl ODS RP-18
Column: 100 × 4.6 3 μm Adsorbosphere
Mobile phase: MeCN:MeOH:100 mM pH 6.5 sodium acetate buffer 20:60:20
Flow rate: 1
Injection volume: 5
Detector: E, ESA Coulochem Model 5100A, Model 5010 analytical cell, porous graphite electrodes +0.6 V

CHROMATOGRAM
Retention time: 8.71
Limit of detection: 0.78 nM

OTHER SUBSTANCES
Simultaneous: ursodiol

KEY WORDS
derivatization

REFERENCE
Bousquet,E.; Santagati,N.A.; Tirendi,S. Determination of chenodeoxycholic acid in pharmaceutical preparations of ursodeoxycholic acid by high performance liquid chromatography with coulometric electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 757–770.

SAMPLE
Matrix: formulations
Sample preparation: Powder capsule contents, weigh out amount containing 25.5 mg chenodiol, add 100 mL acetone:MeCN 40:60, stir for 10 min, filter (paper), dilute with MeCN to a final concentration of 25.5 μg/mL. Mix 500 μL of this solution with 300 μL 1.28 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN, add 50 μL 3% triethylamine in MeCN, heat at 70° for 30 min, cool, add 50 μL 28 μg/mL IS in MeCN, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil RP-18
Mobile phase: Gradient. MeCN:water from 55:45 to 80:20 over 20 min, maintain at 80:20 for 10 min, return to initial conditions over 10 min. Alternatively, isocratic at MeCN:water 70:30.
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 18 (gradient), 7.5 (isocratic)
Internal standard: 6-methoxynaphthacyl ester of valproic acid (Dissolve 2 mmole valproic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μL triethylamine, heat to 60° for 30 min, cool, add 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the organic layers and wash them with 5% sodium bicarbonate solution, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from water to give 6-methoxynaphthacyl ester of valproic acid (mp 56-7°).) (5 (isocratic))
Limit of detection: 2-3 pmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, lithocholic acid

KEY WORDS
derivatization; capsules

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P. 2-Bromoacetyl-6-methoxynaphthalene: A useful fluorescent labelling reagent for HPLC analysis of carboxylic acids, *Chromatographia*, **1992**, *33*, 13–18.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of solution (or hydrolyzed bile) with a 50% molar excess of triethylamine in MeCN, warm briefly, add a 50% molar excess of 100 mM 2-bromoacetophenone in MeCN, heat at 80-90° for 45-60 min, evaporate to dryness, reconstitute with dioxane (Caution! Dioxane is a carcinogen!), filter (0.47 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10/25 ODS

Mobile phase: Gradient. n-Heptane:dioxane 90:10 for 3 min then n-heptane:dioxane:isopropanol 70:25:5 (step gradient). (Caution! Dioxane is a carcinogen!)
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 18
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid, hyodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS
derivatization

REFERENCE
Stellaard,F.; Hachey,D.L.; Klein,P.D. Separation of bile acids as their phenacyl esters by high-pressure liquid chromatography, *Anal.Biochem.*, **1978**, *87*, 359–366.

SAMPLE
Matrix: solutions
Sample preparation: Treat a solution in MeOH with a slight excess of tetramethylammonium hydroxide in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with MeCN, add a 2-10 fold excess of 9-(chloromethyl)anthracene in cyclohexane, heat at 75° for 15 min, very dilute solutions may require longer times), dilute with MeCN, inject an aliquot.

HPLC VARIABLES
Column: 300 mm long "Fatty Acid" reversed-phase (Waters)
Mobile phase: MeOH:water 88:12 (A) or 82:18 (B)
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Simultaneous: cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid
Interfering: deoxycholic acid

KEY WORDS
derivatization

REFERENCE
Korte,W.D. 9-(Chloromethyl)anthracene: a useful derivatizing reagent for enhanced ultraviolet and fluorescence detection of carboxylic acids with liquid chromatography, *J.Chromatogr.*, **1982**, *243*, 153–157.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 100 μL aliquot of a 1-100 μM solution in 20 mM pH 7.1 phosphate buffer with 10 μL 10 mM tetrabutylammonium hydrogen sulfate in water and 100 μL 1 mM N-(9-acridinyl)bromoacetamide in chloroform, stir at 90° for 20-30 min, cool. Remove a 10 μL aliquot of the organic layer and evaporate it to dryness under reduced pressure, reconstitute with mobile phase, sonicate, inject an aliquot. (Synthesis of N-(9-acridinyl)bromoacetamide is as follows. Dissolve 2.49 g 9-aminoacridine hydrochloride

hydrate in water, add dilute NaOH to precipitate the free base, extract with ethyl acetate, dry over anhydrous magnesium sulfate, filter, evaporate to give 9-aminoacridine as yellow needle-shaped crystals (mp 239-240°). Add 1.01 g bromoacetyl bromide in 20 mL diethyl ether dropwise with stirring to 970 mg 9-aminoacridine dissolved in 50 mL acetone containing 1.02 g triethylamine, filter, wash the solid with acetone. Evaporate the filtrate and chromatograph the residue on a 260 × 30 glass column of 70-230 mesh silica gel 60 (Merck) with chloroform:ethyl acetate 2:1. Collect the strong yellow band and evaporate it to dryness, recrystallize from MeOH to give N-(9-acridinyl)bromoacetamide as light yellow crystals (mp 180-182° d).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:water:phosphoric acid 40:60:0.2
Flow rate: 1
Injection volume: 20
Detector: F ex 357.5 em 482

CHROMATOGRAM
Retention time: 36
Limit of detection: 10 fmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid

KEY WORDS
derivatization

REFERENCE
Allenmark,S.; Chelminska-Bertilsson,M.; Thompson,R.A. N-(9-Acridinyl)-bromoacetamide -A powerful reagent for phase-transfer-catalyzed fluorescence labeling of carboxylic acids for liquid chromatography, *Anal.Biochem.*, **1990**, *185*, 279–285.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 1 mg/mL.

HPLC VARIABLES
Column: 100 mm long 5 μm C18
Mobile phase: MeOH:10 mM KH_2PO_4 65:35, pH 7.0
Flow rate: 1
Injection volume: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: k' = 8.03

OTHER SUBSTANCES
Simultaneous: ursodiol

REFERENCE
Roda,A.; Minutello,A.; Angellotti,M.A.; Fini,A. Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partition coefficient and reverse phase HPLC, *J.Lipid Res.*, **1990**, *31*, 1433–1443.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a solution in MeCN:0.8 M NaOH 8:92, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: CarboPac PA-100 (Dionex)
Column: 250 × 4 8.5 μm CarboPac PA-100 (Dionex)
Mobile phase: MeCN:water 15:85 containing 900 mM sodium acetate and 100 mM NaOH
Flow rate: 0.8
Injection volume: 25
Detector: E, Dionex PAD-2 pulsed amperometric detector, gold working electrode, V1 + 0.05 V, t1 480 ms, V2 + 0.60 V, t2 120 ms, V3 -0.60 V, t3 60 ms

CHROMATOGRAM

Retention time: 6.70

OTHER SUBSTANCES

Simultaneous: ursodiol, deoxycholic acid, cholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, glycodeoxychenodeoxycholic acid, ursodeoxycholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, glycolithocholic acid, lithocholic acid, taurolithocholic acid

REFERENCE

Chaplin,M.F. Analysis of bile acids and their conjugates using high-pH anion-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.B*, **1995**, *664*, 431–434.

SAMPLE

Matrix: solutions
Sample preparation: Mix 200 μL of a solution of bile acids with 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, add 300 μL 10 mM tetrakis(decyl)ammonium bromide in 100 mM pH 7.0 phosphate buffer, heat at 40° for with sonication 10 min, add 300 μL 5.1 μM IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 Ultracarb 5 ODS
Mobile phase: Gradient. A was water. B was MeCN:MeOH 60:40. A:B 55:45 for 20 min, to 30:70 over 10 min, maintain at 30:70 for 25 min, return to initial conditions over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 29
Internal standard: 6-methoxynaphthacyl ester of lauric acid (36)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Simultaneous: cholic acid, deoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization

REFERENCE

Gatti,R.; Roda,A.; Cerre,C.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum, *Biomed.Chromatogr.*, **1997**, *11*, 11–15.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a solution in MeOH with 100 μL 2.5 μg/mL IS in MeOH, evaporate to dryness, add 150 μL 0.2% 3-[4-(bromomethyl)phenyl]-7-(diethylamino)-2H-1-benzopyran-2-one (3-(4-bromomethylphenyl)-7-diethylaminocoumarin) in MeCN, add crown ether solution, heat at 60° for 20 min, evaporate to dryness at room temperature, reconstitute with 500 μL chloroform, add to a 55 × 6 column of silica gel, wash with 6 mL chloroform:MeOH 200:1, elute with 5 mL chloroform:MeOH 8:1, evaporate to dryness, reconstitute with 200 μL MeOH, inject a 5 μL aliquot. (Prepare crown ether solution by adding a large excess of solid potassium bicarbonate to a 0.4% 18-crown-6 solution in MeCN, sonicate at room temperature for 10 min, centrifuge at 1000 g for 10 min, use the supernatant. Synthesize 3-[4-(bromomethyl)phenyl]-7-(diethylamino)-2H-1-benzopyran-2-one as follows. Add 18.8 g aluminum trichloride to a solution of 94 mmoles m-diethylaminophenol and 84 g triethyl orthoformate in 185 mL chloroform at room temperature, mix for 10 min, add 50 mL 10% HCl, stir, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform. Wash the organic layer with saturate NaCl and dry it over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure, recrystallize the product from chloroform to give 4-(diethylamino)-2-hydroxybenzaldehyde (Bull.Chem.Soc.Jpn. 1985, 58, 2192). Reflux 90 g α-bromo-p-toluic acid and 25 mL concentrated sulfuric acid in 1 L MeOH for 75 min, concentrate to 200 mL under reduced pressure, dilute with 3 mL water, recrystallize from MeOH to give methyl α-bromo-p-toluate as colorless needles (mp 54-55°). Add 35 g NaCN in 85 mL water over 10 min to a stirred solution of 70 g methyl α-bromo-p-toluate in 350 mL MeOH, heat at 50° for 30 min, dilute to 1 L with cold water, filter, recrystallize from light petroleum:benzene 2:1 (Caution! Benzene is a carcinogen!) with the aid of charcoal to give methyl α-cyano-p-toluate as colorless needles (mp 63-64°). Reflux 500 mg methyl α-cyano-p-toluate in 12 mL 10% NaOH in water for 30 min, filter, acidify the filtrate. Collect the precipitate and recrystallize it from glacial acetic acid to give 4-carboxyphenylacetic acid (homoterephthalic acid) (mp 239-241°) (J. Org. Chem. 1952, 17, 1035). Reflux 4-carboxyphenylacetic acid in MeOH in the presence of sulfuric acid to obtain methyl 4-carbomethoxyphenyl acetate (bp 172-175°/20 mm Hg) (J. Indian Chem. Soc. 1987, 64, 34). Heat 8.69 g 4-(diethylamino)-2-hydroxybenzaldehyde, 15.6 g methyl 4-carbomethoxyphenyl acetate, 2 mL piperidine, and 68 mL pyridine at 100° for 15 h, evaporate to dryness, recrystallize from ethyl acetate/hexane to give methyl 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoate as reddish-yellow prisms (mp 179-180.5). (A second crop can be obtained from the mother liquor by chromatography on silica gel using chloroform:hexane:acetone 20:18:1.) Suspend 7.1 g methyl 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoate in 240 mL 6 M HCl, reflux for 19 h, evaporate most of the solvent under reduced pressure, neutralize the residue with a saturated aqueous solution of sodium bicarbonate, filter, recrystallize from acetone to give 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoic acid as reddish-yellow prisms (mp 282-283.5°). Add 2.2 mL ethyl chloroformate and 3.2 mL triethylamine to a solution of 1.69 g 4-[7-(diethylamino)-2-oxo-2H-1-benzopyran-3-yl]benzoic acid in 200 mL THF, stir at room temperature for 1 h, add a solution of 2.27 g sodium borohydride in 4.8 mL water over 30 min, stir for 1 h, acidify with acetic acid, evaporate the THF under reduced pressure, add chloroform and water to the residue. Remove the organic layer and wash it with water, dry over anhydrous magnesium sulfate, evaporate to dryness, chromatograph on silica gel with chloroform, recrystallize from ethyl acetate/hexane to give 7-(diethylamino)-3-[4-(hydroxymethyl)phenyl]-2H-1-benzopyran-2-one as yellow needles (mp 153-154°). Stir 3.23 g 7-(diethylamino)-3-[4-(hydroxymethyl)phenyl]-2H-1-benzopyran-2-one and 86 mL phosphorus tribromide at 40-50° for 3 days, pour into ice-water, filter. Dissolve the solid in chloroform and wash with saturated aqueous sodium bicarbonate, wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from ethyl acetate/hexane to give 3-[4-(bromomethyl)phenyl]-7-(diethylamino)-2H-1-benzopyran-2-one as yellow needles (mp 166-167°).)

HPLC VARIABLES
Column: 250 × 4.6 Inertsil C8
Mobile phase: Gradient. A was MeOH:20 mM pH 7.5 Tris-acetate buffer 78:22. B was MeOH:20 mM pH 7.5 Tris-acetate buffer 90:10. A:B 100:0 for 20 min, to 65:35 over 30 min, to 35:65 (step gradient), to 10:90 over 20 min.
Flow rate: 1
Injection volume: 5
Detector: F ex 400 em 475

CHROMATOGRAM
Retention time: 52
Internal standard: 3α,7α,12α-trihydroxy-26a,26b-dihomo-27-nor-5β-cholestan-26b-oic acid (60)
Limit of detection: 15 fmole

OTHER SUBSTANCES
Simultaneous: numerous other bile acids, cholic acid

KEY WORDS
derivatization

REFERENCE
Kurosawa,T.; Sato,H.; Sato,M.; Takechi,H.; Machida,M.; Tohma,M. Analysis of stereoisomeric C_{27}-bile acids by high-performance liquid chromatography with fluorescence detection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1375–1382.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, pass 40 mL urine through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 10 mL 100 mM pH 5.0 sodium acetate buffer, add 100 μg β-glucuronidase, add 100 μg cholylglycine hydrolase, heat at 37° for 36 h, pass the mixture through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 85 μm μBondapak C18 radial pack
Mobile phase: MeCN:MeOH:water:acetic acid 70:20:70:1
Flow rate: 2
Injection volume: 50
Detector: RI

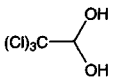
CHROMATOGRAM
Retention time: 32
Limit of detection: 1000 ng

OTHER SUBSTANCES
Extracted: ursodiol, bile acids, deoxycholic acid

KEY WORDS
SPE

REFERENCE
Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, *26*, 690–698.

Chloral hydrate



Molecular formula: $C_2H_3Cl_3O_2$
Molecular weight: 165.40
CAS Registry No.: 302-17-0
Merck Index: 2113

SAMPLE

Matrix: blood
Sample preparation: 250 µL Plasma + 250 µL 150 µg/mL 4-chloro-1-butanol in water, vortex, add 20 µL benzoyl chloride dropwise on the surface, vortex, add 250 µL 4 M NaOH, rotate at medium sped for 10 min, add 10 mL pentane, rotate for 5-7 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness at 45-50°, reconstitute the residue in 100 µL MeOH, inject a 5-10 µL aliquot.

HPLC VARIABLES

Guard column: 7 µm RP-18 (Brownlee)
Column: 150 × 4.6 5 µm Ultrasphere ODS
Mobile phase: MeCN:MeOH:water 30:30:40
Flow rate: 2
Injection volume: 5-10
Detector: UV 237

CHROMATOGRAM

Retention time: 12 (for trichloroethanol, the active metabolite)
Internal standard: 4-chloro-1-butanol (7)
Limit of quantitation: 7000 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, salicylic acid, barbiturates

KEY WORDS

plasma; derivatization

REFERENCE

Gupta,R.N. Determination of trichloroethanol, the active metabolite of chloral hydrate, in plasma by liquid chromatography, *J.Chromatogr.*, **1990**, *500*, 655–659.

SAMPLE

Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 200 µL 3 mg/mL 2,4-dinitrophenylhydrazine in 2 M HCl (freshly prepared), shake for 10 min, extract twice with 1 mL portions of ethyl acetate. Combine the extracts and evaporate them to dryness under reduced pressure, reconstitute with 500 µL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil ODS
Mobile phase: MeCN:water 55:45
Flow rate: 2
Detector: UV 330

CHROMATOGRAM

Retention time: 11.1

OTHER SUBSTANCES

Extracted: acetaldehyde, acetone, 9-chloroanthracene, formaldehyde, malondialdehyde, propionaldehyde

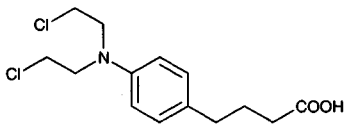
KEY WORDS

mouse; liver; derivatization

REFERENCE

Ni,Y.-C.; Wong.T.-Y.,; Lloyd,R.V.; Heinze,T.M.; Shelton,S.; Casciano,D.; Kadlubar,F.F.; Fu,P.P. Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol leading to induction of lipid peroxidation via a free radical mechanism, *Drug Metab.Dispos.*, **1996**, *24*, 81–90.

Chlorambucil



Molecular formula: $C_{14}H_{19}Cl_2NO_2$
Molecular weight: 304.22
CAS Registry No.: 305-03-3
Merck Index: 2116

SAMPLE

Matrix: blood
Sample preparation: Directly inject a 20-30 μL aliquot of plasma.

HPLC VARIABLES

Guard column: 50 × 3.9 30-32 μm pellicular Co:Pell ODS
Column: 250 × 4.6 10 μm PXS-10/25 ODS (A) or 150 × 4.6 5 μm Chromegabond MC-18 (B)
Mobile phase: MeOH:20 mM KH_2PO_4 50:50 (A) or 55:45 (B)
Flow rate: 1.5 (A), 1.0 (B)
Injection volume: 20-30
Detector: UV 254, UV 280, F ex 285 em 320 (cut-off filter)

CHROMATOGRAM

Retention time: 15 (A), 9 (B)
Limit of detection: 11.9 pmole

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; direct injection

REFERENCE

Zakaria,M.; Brown,P.R. Rapid assay for plasma chlorambucil and phenyl acetic mustard using reversed-phase liquid chromatography, *J.Chromatogr.*, **1982**, *230*, 381–389.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL 2 μg/mL IS in MeOH to 100 μL plasma, mix, centrifuge at -20° at 4500 g for 15 min. Remove the supernatant and dry it under vacuum. Resuspend the residue in mobile phase, centrifuge at 4° at 200 g for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18 Guard-Pak
Column: 100 × 8 4 μm Nova-Pak C18 Radial-Pak
Mobile phase: MeOH:water:100 mM tetrabutylammonium hydroxide in water 70:25:5, adjusted to pH 7.4 with orthophosphoric acid
Flow rate: 3
Injection volume: 10
Detector: U 254, UV 280

CHROMATOGRAM

Retention time: 3.5
Internal standard: β mono(trifluoromethyl) analogue (4.8)
Limit of detection: 60 ng/mL

KEY WORDS

mouse; plasma

REFERENCE

Workman,P.; Oppitz,M.; Donaldson,J.; Lee,F.Y. High-performance liquid chromatography of chlorambucil analogues, *J.Chromatogr.*, **1987**, *422*, 315–321.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 10.00

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen;

tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Mix 250 μL blood with 2 volumes of cold MeCN:DMSO 95:5. Centrifuge, dilute with 2 volumes of mobile phase, inject a 20 μL aliquot. Tissue. Homogenize tissue with 2 volumes of cold MeCN. Centrifuge the homogenate, dilute with 2 volumes of mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: Hypersil ODS-2 C18
Column: 100 × 4.0 Hypersil ODS-2 C18
Mobile phase: MeCN:acetic acid:water 72.5:0.5:27
Flow rate: 0.6
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 90 ng/mL

KEY WORDS

lung; liver; kidney; human; dog; rat; rabbit; pharmacokinetics

REFERENCE

Saah,F.; Wu,W.-M.; Eberst,K.; Marvanyos,E.; Bodor,N. Design, synthesis, and pharmacokinetic evaluation of a chemical delivery system for drug targeting to lung tissue, *J.Pharm.Sci.*, **1996**, *85*, 496–504.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Plasma + 100 μL phenylpropionic mustard and chlorambucil propyl ester in MeOH + 3 mL MeCN + 1 mL dichloromethane + 2 mL hexane, shake, centrifuge at 2000 g at 4° for 10 min. Remove the top two organic layers and evaporate them to dryness, reconstitute the residue in 200 μL MeOH:hexane 3:1, inject an aliquot. Tissue. Tissue + 5 mL chilled MeCN + 100 μL phenylpropionic mustard and chlorambucil propyl ester in MeOH, sonicate for 30 s on ice, shake vigorously, centrifuge at 2000 g at 4° for 10 min. Remove the top organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeOH:hexane 3:1, inject an aliquot. (Final concentrations of phenylpropionic mustard should be 2.5 μg/mL and 0.25 μg/g and of chlorambucil propyl ester should be 2.5 μg/mL and 2.5 μg/g in plasma and tissue respectively.)

HPLC VARIABLES

Guard column: pellicular C18 (Waters)
Column: 100 × 4.6 5 μm RAC III Partisil 5 ODS 3
Mobile phase: Gradient. A was water:acetic acid 98:2. B was MeCN:acetic acid 98:2. A:B from 30:70 to 65:35 (sic) over 17 min (Waters curve no. 7), maintain at 65:35 for 7 min

Flow rate: from 1.5 to 2.5 over 17 min, maintain at 2.5 for 7 min
Detector: UV 254

CHROMATOGRAM
Retention time: 13.7
Internal standard: phenylpropionic mustard (12.6), chlorambucil propyl ester (19.0)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: phenylacetic mustard, chlorambucil tert-butyl ester

KEY WORDS
plasma; pharmacokinetics; rat; brain

REFERENCE
Greig,N.H.; Stahle,P.L.; Shetty,H.U.; Genka,S.; John,V.; Soncrant,T.T.; Rapoport,S.I. High-performance liquid chromatographic analysis of chlorambucil tert.-butyl ester and its active metabolites chlorambucil and phenylacetic mustard in plasma and tissue, *J.Chromatogr.*, **1990**, *534*, 279–286.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM
Retention time: 22.387

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Add microsomal incubation to 2 volumes methyl ethyl ketone, vortex for 45 s, centrifuge at 100 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 1 mL MeOH, filter (0.2 μm nylon), inject an aliquot.

HPLC VARIABLES

Column: 150 × 2 4 μm Nova Pak C18
Mobile phase: MeOH:0.05% trifuoroacetic acid, pH 2.25 52:48
Flow rate: 0.35
Detector: UV 258 for 15 min then UV 249

CHROMATOGRAM

Retention time: 32
Internal standard: 3-(4-hydroxyphenyl)propionic acid (8)
Limit of quantitation: 40 ng

OTHER SUBSTANCES

Extracted: chlorambucil N-oxide

KEY WORDS

rat; liver

REFERENCE

Chandler,K.J.; McCabe,J.B.; Kirkpatrick,D.L. High-performance liquid chromatographic method for the separation of chlorambucil and its N-oxide prodrug, *J.Chromatogr.B*, **1994**, *652*, 195–202.

SAMPLE

Matrix: reaction mixtures
Sample preparation: Mix an aliquot with an equal volume of 20 mM pH 4.4 KH_2PO_4, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:20 mM pH 4.4 KH_2PO_4 65:35
Flow rate: 1
Injection volume: 20
Detector: UV 254

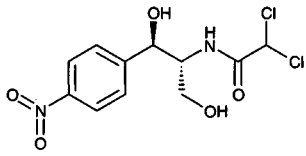
CHROMATOGRAM

Retention time: 13.2
Limit of detection: 750 ng/mL

REFERENCE

Lunn,G.; Sansone,E.B.; Andrews,A.W.; Hellwig,L.C. Degradation and disposal of some antineoplastic drugs, *J.Pharm.Sci.*, **1989**, *78*, 652–659.

Chloramphenicol



Molecular formula: $C_{11}H_{12}Cl_2N_2O_5$
Molecular weight: 323.13
CAS Registry No.: 56-75-7
Merck Index: 2120

SAMPLE

Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254, UV 280

CHROMATOGRAM

Retention time: 9.5
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 2 μg/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefotaxime, cefoxitin, cephapirin
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS

serum

REFERENCE

Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 1 mL 1 M pH 3.0 sodium acetate + 5 mL diethyl ether, shake for 20 min, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness at 40° under a stream of nitrogen. Reconstitute the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM potassium acetate buffer 20:80, pH 6.5
Flow rate: 1.6
Injection volume: 50
Detector: UV 215

CHROMATOGRAM

Retention time: 5.10

Internal standard: chloramphenicol

OTHER SUBSTANCES

Simultaneous: penicillin V
Noninterfering: amoxicillin, ampicillin, tetracycline, doxycycline, cephalexin, amikacin, sisomicin, netilmicin, tobramycin, gentamicin, phenemal, phenacetin, ethosuximide, primidone, phenytoin, amiloride, hydrochlorothiazide
Interfering: penicillin G procaine, cloxacillin

KEY WORDS

serum; chloramphenicol is IS

REFERENCE

Lindberg,R.L.; Huupponen,R.K.; Huovinen,P. Rapid high-pressure liquid chromatographic method for analysis of phenoxymethylpenicillin in human serum, *Antimicrob.Agents Chemother.*, **1984**, *26*, 300–302.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 273

CHROMATOGRAM

Retention time: 3.85
Internal standard: 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: phenacetin

KEY WORDS

serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 4.08
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin
Interfering: butabarbital

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL 220 μg/mL 4-nitroacetanilide in ethyl acetate, vortex for 30 s, centrifuge at 7000 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: Isopropanol:100 mM pH 5.0 sodium acetate buffer 2:98
Flow rate: 2

Injection volume: 10
Detector: UV 278

CHROMATOGRAM
Retention time: 6.32
Internal standard: 4-nitroacetanilide (8.27)
Limit of quantitation: 10000 ng/mL

OTHER SUBSTANCES
Extracted: caffeine
Simultaneous: theobromine, theophylline, diphylline, chloramphenicol 3-monosuccinate
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, carbamazepine, cyclosporine, digoxin, desipramine, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum

REFERENCE
Markin,R.S.; Wadman,M.C.; Bottjen,P.L.; Haven,M.C.; Huth,J.A. Short-column liquid chromatographic assay for caffeine and chloramphenicol in serum, *J.Chromatogr.*, **1990**, *525*, 464–470.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 20 μg/mL hydroxyethyltheophylline in 2 M perchloric acid, vortex, centrifuge 5 min, inject 50 μL aliquot of supernatant.

HPLC VARIABLES
Column: 125 × 4 LiChroSpher RP-8 5 μm
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mL 2 M sodium acetate + 845 mL water, pH adjusted to 4.0 with acetic acid.)
Column temperature: 45
Flow rate: 1.5
Injection volume: 50
Detector: UV 282

CHROMATOGRAM
Retention time: 18
Internal standard: hydroxyethyltheophylline (5.6)

OTHER SUBSTANCES
Simultaneous: theophylline, caffeine

KEY WORDS
serum

REFERENCE
Hannak,D.; Haux,P.; Scharbert,F.; Kattermann,R. Liquid chromatographic analysis of phenobarbital, phenytoin, and theophylline, *Wien.Klin.Wochenschr.Suppl.*, **1992**, *191*, 27–31.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column

A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A: B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 10.52
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A: B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 9.92
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, urine
Sample preparation: 200 μL Plasma (dog) or urine (rat) + 50 μL ammonium hydroxide: water 50:50 + 1 mL MTBE, extract, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 3.65 μm Zorbax Rx-SIL (similar to Zorbax SB-C8 (Mac-Mod Analytical))
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.1% trifluoroacetic acid adjusted to pH 3 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 20
Detector: UV 278

CHROMATOGRAM
Retention time: 4

KEY WORDS
plasma; dog; rat

REFERENCE
Kirkland,K.M.; McCombs,D.A.; Kirkland,J.J. Rapid, high-resolution high-performance liquid chromatographic analysis of antibiotics, *J.Chromatogr.A*, **1994**, *660*, 327–337.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.105

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: feces
Sample preparation: Homogenize (Polytron) with three volumes water, centrifuge at 4° at 23000 g for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 200 × 4.6 5 μm ODS2 (SFCC)
Mobile phase: MeOH:100 mM pH 4.4 citrate buffer 20:80
Flow rate: 0.8
Detector: UV 280

CHROMATOGRAM
Retention time: 57.9

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
duck; radiolabeled

REFERENCE
Cravedi,J.P.; Baradat,M.; Debrauwer,L.; Alary,J.; Tulliez,J.; Bories,G. Evidence for new metabolic pathways of chloramphenicol in the duck, *Drug Metab.Dispos.*, **1994**, *22*, 578–583.

SAMPLE
Matrix: formulations
Sample preparation: Add capsule contents to water, extract with three 20 mL portions of ethyl acetate. Dry the extracts over potassium carbonate and evaporate them to dryness under reduced pressure. Add 120 mg of this product to 2.5 mL 5% HCl, heat at 95° for 2.5 h, cool to room temperature, adjust pH to 11 with concentrated NaOH, extract five times with 7 mL portions of ethyl acetate. Combine the extracts and dry them over potassium carbonate, evaporate to dryness, prepare a 10 mg/mL solution in MeOH. Add a 50 μL aliquot to 150 μL 10 mg/mL 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT) in MeCN, let stand at room temperature for 20 min, add 2 μL 2-aminoethanol, let stand for 10 min, add 50 μL 300 mM pH 3 ammonium phosphate, inject a 2-5 μL aliquot. (Chloramphenicol is de-acylated to 2-amino-1-(p-nitrophenyl)-1,3-propanediol which is then derivatized. The diastereomer obtained from chloramphenicol (1R,2R) can be resolved from the other possible diastereomers.)

HPLC VARIABLES
Column: 150 × 3.9 Nova-pak ODS
Mobile phase: MeOH:water 42:58
Flow rate: 1
Injection volume: 2-5
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (1R,2R), 18 (1S,2S), 22 (1R,2S or 1S,2R) , 25 (1R,2S or 1S,2R)

KEY WORDS
derivatization; capsules

REFERENCE
Gal,J.; Meyer-Lehnert,S. Reversed-phase liquid chromatographic separation of enantiomeric and diastereomeric bases related to chloramphenicol and thiamphenicol, *J.Pharm.Sci.*, **1988**, *77*, 1062–1065.

SAMPLE
Matrix: milk, tissue
Sample preparation: Prepare a SPE extraction column by placing 1.2 g silica gel (40 μm, Baker) in a 3 mL filtration column (Baker), condition with 8 mL ethyl acetate:hexane 40:60, do not allow to dry. Tissue. 10 g Ground tissue + 20 mL ethyl acetate, stir thoroughly with a glass rod, sonicate below 40° for 15 min, allow to settle, decant through 5 g anhydrous sodium sulfate on filter paper (S & S 589.1), repeat extraction, wash solids with 10 mL ethyl acetate. Combine all organic layers, add 60 mL hexane, stir thoroughly, allow to stand for 5 min, filter (S & S 589.3 paper), wash filter with 10 mL hexane. Combine all organic layers and pass through the SPE column at 8/10 mL/min, wash with 10 mL hexane, dry in a stream of nitrogen for 20 min, elute with four 1 mL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 50 μL aliquot. Milk. 10 g Homogenized milk + 0.4 g citric acid monohydrate + 30 mL ethyl acetate, shake mechanically for 15 min, centrifuge at 2300 g for 10 min, filter (paper) the organic layer, repeat extraction with 10 mL ethyl acetate, filter (paper), wash filter with 10 mL ethyl acetate, add 60 mL hexane to filtrate, stir thoroughly, allow to stand for 5 min, filter (S & S 589.3 paper), wash filter with 10 mL hexane. Combine all organic layers and pass through the SPE column at 8/10 mL/min, wash with 10 mL hexane, dry in a stream of nitrogen for 20 min, elute with four 1 mL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 50 μL aliquot. (J.Chrom. 1991, 566, 173)

HPLC VARIABLES
Guard column: 10 × 2.1 reversed-phase (Chrompack)
Column: Two 100 × 3 5 μm ChromSpher C8 glass columns in series (Chrompack)
Mobile phase: MeCN:10 mM pH 4.3 sodium acetate buffer 25:75
Flow rate: 0.8
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 1 ng/g (milk), 10 ng/g (tissue)

KEY WORDS
pig; cow; muscle; SPE

REFERENCE
Haagsma,N.; Schreuder,C.; Rensen,E.R.A. Rapid sample preparation method for the determination of chloramphenicol in swine muscle by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *363*, 353–359.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES

Guard column: Guard-PAK C18 (Waters)
Column: 150 × 3.9 5 μm NOVA PAK C18
Mobile phase: MeOH:50 mM pH 6.0 KH_2PO_4 50:50
Flow rate: 0.6
Detector: UV 313

CHROMATOGRAM

Retention time: 4.2

REFERENCE

Koga,H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbu-

tazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 3 mL silica SPE cartridge with 10 mL ethyl acetate:hexane (4:10 ?). Vortex 3 g fatty liver and 3 mL water for 1 min, add 15 mL hexane, stir at 40 rpm for 10 min. Centrifuge at 2400 g for 5 min and discard the supernatant. Add 12 mL ethyl acetate, vortex twice for 1 min periods at a 15 min interval, centrifuge at 4000 g for 5 min, decant 8 mL organic phase through glass wool covered by 1 g anhydrous sodium sulfate, wash twice with 2 ml portions of ethyl acetate. Add 30 mL hexane to the combined extracts. Add to the SPE cartridge, rinse the flask and sodium sulfate with 10 mL ethyl acetate:hexane 4:10, add the rinse to the SPE cartridge, wash with 10 mL hexane, dry under vacuum for 2 min, elute with 5 mL 50 mM pH 10 potassium phosphate buffer. Add 20 mL ethyl acetate to the eluate, vortex, centrifuge at 2400 g for 10 min. Evaporate 18 mL of the ethyl acetate phase to dryness under reduced pressure, reconstitute the oily residue in 1.2 mL hexane:chloroform 50:50 (Caution! Chloroform is a carcinogen!). Add 800 μL water, stir at 35 rpm for 5 min, centrifuge at 3300 g for 10 min, inject an aliquot.

HPLC VARIABLES

Guard column: 4 × 4 4 μm Nova-Pak C18
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:5 mM pH 7.9 diammonium hydrogen phosphate buffer 19:81
Flow rate: 1
Detector: UV 278

CHROMATOGRAM

Retention time: 8.7
Limit of detection: 400 pg/g
Limit of quantitation: 800 pg/g

KEY WORDS

SPE; liver

REFERENCE

Roudaut,B. High-performance liquid chromatography with UV detection and scanning UV confirmation of chloramphenicol in fatty liver, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1097–1105.

SAMPLE
Matrix: tissue
Sample preparation: Grind muscle, liver or kidney with a kitchen mixer for 1 min. Weigh out 5 g ground tissue, add 2 mL water, vortex for 1 min, let stand for 10 min, add 6 mL ethyl acetate, vortex for 1 min, centrifuge at 4000 rpm for 5 min. Remove 4.2 mL of the upper organic layer and evaporate it to dryness under reduced pressure at 30°, suspend the residue in 1.4 mL hexane:chloroform 50:50, add 0.7 mL water, stir (Heidolf stirrer) at 35 rpm for 5 min, centrifuge at 4000 rpm for 10 min, repeat if necessary, inject a 200 µL aliquot of the supernatant

HPLC VARIABLES
Guard column: 4 × 4 C8
Column: 150 × 4.6 Novapack C18
Mobile phase: MeCN:0.66 g/L pH 7.9 $(NH_4)_2HPO_4$ 29:71 (muscle) or 18:82 (liver) or MeCN: 0.66 g/L $(NH_4)_2HPO_4$ adjusted to pH 8.3 with 25% ammonia solution 29:71 (kidney) (Flush daily with MeCN:0.1% sulfuric acid 18:82.)
Flow rate: 1
Injection volume: 200
Detector: UV 278

CHROMATOGRAM
Limit of detection: 1 ng/g

KEY WORDS
cow; muscle; liver; kidney

REFERENCE
Sanders,P.; Guillot,P.; Dagorn,M.; Delmas,J.M. Liquid chromatographic determination of chloramphenicol in calf tissues: studies of stability in muscle, kidney, and liver, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 483–486.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak silica cartridge with 5 mL MeCN:water 20:80, 5 mL MeCN, and 5 mL dichloromethane, then dry with a stream of nitrogen for 30 min. Homogenize tissue (Sorvall Omnimixer). 10 g Homogenized tissue + 30 g anhydrous sodium sulfate + 30 mL MeCN, homogenize for 1 min, centrifuge at 4000 rpm for 10 min, remove the MeCN layer, extract the aqueous layer twice more with 30 mL MeCN. Combine the MeCN layers and wash them twice with 60 mL portions of n-hexane with vigorous shaking for 30 s each time. Evaporate the MeCN layer to dryness under a stream of nitrogen at 50°, dissolve the residue in dichloromethane, add the sample extract to the SPE cartridge, wash with two 5 mL aliquots of dichloromethane, dry cartridge with a stream of nitrogen for 30 min, elute with 5 mL MeCN:water 20:80. Add the eluate to 1 mL ethyl acetate, shake, remove the upper organic layer, repeat the extraction twice more, combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 4 µm Nova-Pak C18 radial compression module
Mobile phase: MeCN:buffer 30:70 (Prepare buffer by diluting 1 M pH 4.8 sodium acetate buffer 1:100 with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 278

CHROMATOGRAM
Retention time: 7.5

Limit of detection: 10 ppb

KEY WORDS
chicken; muscle; SPE

REFERENCE
Ramos,M.; Reuvers,T.; Aranda,A.; Gómez,J. Determination of chloramphenicol in chicken muscle by high performance liquid chromatography and UV-diode array detection, *J.Liq.Chromatogr.*, **1994**, *17*, 385–401.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize tissue with an Omni-mixer (Sorval). Sonicate 10 g muscle or 5 g liver with 40 mL water for 8 min, centrifuge for 10 min, add 20 mL of the supernatant to a Chem Elut CE 1020 SPE cartridge (Analytichem), allow to equilibrate for 15 min, elute with 50 mL ethyl acetate. Evaporate the eluate to dryness under educed pressure, reconstitute with 500 μL water, add 1 mL toluene, vortex gently, centrifuge, discard the organic layer, repeat wash. Filter (0.45 μm) the aqueous phase, inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil RP-18
Mobile phase: MeCN:50 mM pH 3 triethylamine phosphate buffer 21:79
Flow rate: 1
Injection volume: 100
Detector: UV 278

CHROMATOGRAM
Retention time: 11
Limit of detection: 2 ng/g
Limit of quantitation: 8 ng/g

KEY WORDS
liver; muscle; quail; pheasant; mallard; SPE

REFERENCE
Di Pietra,A.M.; Piazza,V.; Andrisano,V.; Cavrini,V. HPLC determination of chloramphenicol and thiamphenicol residues in gamebird meats, *J.Liq.Chromatogr.*, **1995**, *18*, 3529–3543.

SAMPLE
Matrix: tissue
Sample preparation: Dry 10 g homogenized (Ultra-Turrax) tissue and 5 g sand at 60° for 5 h, mix with 20 mL MeCN, homogenize (Ultra-Turrax) for 3 min, centrifuge at 2980 g for 5 min, decant the supernatant, repeat the extraction. Combine the extracts and evaporate them to dryness, dissolve the residue in 1 mL MeCN, add to a Sep-Pak C18 cartridge, rinse tube twice with 1 mL portions of MeCN, add rinses to the SPE cartridge, elute with 4 mL MeCN. Evaporate the eluate to dryness and reconstitute the residue in ethyl acetate, pass through a silica SPE cartridge, elute with ethyl acetate. Evaporate the eluate to dryness under reduced pressure, reconstitute in 500 μL n-hexane and 500 μL mobile phase, inject a 50 μL aliquot of the lower aqueous phase onto column A and elute to waste with mobile phase at 0.7 mL/min, divert the fraction containing chloramphenicol onto column B while eluting column B to waste. When all the chloramphenicol has eluted from column A remove column A from the circuit, backflush the contents of column B onto column C with mobile phase at 0.9 mL/min, monitor the effluent from column C.

HPLC VARIABLES

Column: A 150 × 4.6 5 μm LC-HISEP (Supelco); B 150 × 4.6 5 μm Supelcosil LC-18; C 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:THF:water 80:2:18
Flow rate: 0.7, 0.9
Injection volume: 50
Detector: UV 278

CHROMATOGRAM

Retention time: 26
Limit of quantitation: 2 ng/g

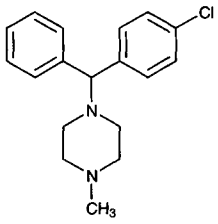
KEY WORDS

column-switching; pig; liver; kidney; muscle; fat; skin; turkey; trout; fish; SPE

REFERENCE

Hummert,C.; Luckas,B.; Siebenlist,H. Determination of chloramphenicol in animal tissue using high-performance liquid chromatography with a column-switching system and ultraviolet detection, *J.Chromatogr.B*, **1995**, *668*, 53–58.

Chlorcyclizine



Molecular formula: $C_{18}H_{21}ClN_2$
Molecular weight: 300.83
CAS Registry No.: 82-93-9, 1620-21-9 (HCl)
Merck Index: 2128
Lednicer No.: 1 58

SAMPLE

Matrix: blood, CSF
Sample preparation: Plasma. Centrifuge blood at 7000 rpm, decant 100 μL plasma. Mix 100 μL plasma with 200 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25 μL water to 25 μL CSF, mix with 50 μL acetone, centrifuge at 7000 rpm for 5 min, decant the supernatant, evaporate under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:water 32:68 containing 0.05% trifluoroacetic acid

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Chou,K.-J.; Donovan,M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm.Drug Dispos.*, **1997**, *18*, 335–346.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide,

dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 3.59

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(4-tert-butylphenylcarbamate)

Mobile phase: Hexane:isopropanol 98:2
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: 9.5 (+), 10.5 (-)

KEY WORDS
chiral

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, clonidine, diphenhydramine, promethazine, pyrilamine, triprolidine

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REFERENCE
Supelco Catalog, **1994**, 768.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: tripelennamine, triprolidine, cyclizine, methaphenilene, pyrrobutamine, meclizine, buclizine

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REFERENCE
Vydac HPLC Catalog, 1994-5,

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

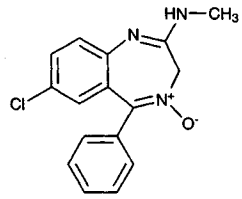
CHROMATOGRAM
Retention time: 14.70 (A), 6.89 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Chlordiazepoxide



Molecular formula: $C_{16}H_{14}ClN_3O$
Molecular weight: 299.76
CAS Registry No.: 58-25-3, 438-41-5 (HCl)
Merck Index: 2132
Lednicer No.: 1 365

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 20 µL 20 µg/mL IS + 200 µL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 µL mobile phase, inject a 20 µL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 µm TSK gel Super-ODS (A) or 100 × 4.6 5 µm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 21.0 (A), 73.1 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 1 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-µm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: 200 µL Serum + 200 µL 50 µg/mL hexobarbital in MeCN + 25 µL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 µL aliquot of the supernatant.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3

Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 19.6
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, butalbital, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane:isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.53
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES

Extracted: amitriptyline, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, diazepam, doxepin, haloperidol, imipramine, nortriptyline, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: desmethyldoxepin, fluphenazine, oxazepam

KEY WORDS

plasma

REFERENCE

Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE

Matrix: blood

Sample preparation: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES

Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm

Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.

Flow rate: 1

Injection volume: 100-200

Detector: UV 230

CHROMATOGRAM

Retention time: 22.8

OTHER SUBSTANCES

Extracted: alprazolam, bromazepam, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam

Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid

KEY WORDS

plasma; column-switching

REFERENCE

Lacroix,C.; Wojciechowski,F.; Danger,P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE

Matrix: blood

Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 × 4.6 LC-8-DB (Supelco)

Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)

Flow rate: 2

Injection volume: 100

Detector: UV 228

CHROMATOGRAM

Retention time: 3.6
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: desipramine, ibuprofen, methaqualone, norverapamil, promazine, propafenone

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 262

CHROMATOGRAM

Retention time: 5.10

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 12.96
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, gastric contents, tissue, urine
Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH: water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES
Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 230, 254

CHROMATOGRAM
Retention time: 14.5

OTHER SUBSTANCES
Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, pindolol, zolpidem
Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone,

methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, saliva, tissue, urine

Sample preparation: Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5 NaH_2PO_4 containing 0.5% NaF. Add 500 μL brain homogenate or 500 μL plasma, saliva, or urine containing 15 μL saturated NaF solution to 75 μL 150 μg/mL IS, add 50 μL 50% perchloric acid, mix vigorously for 10 s, let stand at room temperature for 10 min, add 1 mL water, mix briefly, centrifuge at 10° at 2500 (?) for 30 min. Remove the supernatant and add it to 750 μL saturated sodium carbonate solution, mix briefly, add 7.5 mL pentane:chloroform 95:5, rock gently for 10 min, centrifuge in a desk-top centrifuge for 2 min, freeze in dry ice/acetone for 2 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix vigorously for 10 s, centrifuge in a desk-top centrifuge for 1-2 min, freeze in dry ice/acetone for 3-5 min, discard the organic layer. Allow the aqueous layer to thaw, remove any trace of organic solvent with a stream of nitrogen, inject a 75 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee RP-8

Column: 250 × 4.6 5 μm Zorbax RX-C18

Mobile phase: MeCN:buffer 18:82 (Buffer was 100 mM K_2HPO_4 containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)

Flow rate: 2

Injection volume: 75

Detector: UV 235

CHROMATOGRAM

Retention time: 8.3

Internal standard: 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

OTHER SUBSTANCES

Extracted: clozapine, cocaine, gepirone, methylphenidate, pentazocine, pseudococaine

Simultaneous: acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, benperidol, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hydergine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaquaalude, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylecgonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428

Noninterfering: amitriptyline, benztropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobam-

ate, methadone, methamphetamine, nicotine, norfluoxetine, nortriptyline, PCP, phenothiazine, pseudoephedrine

KEY WORDS
rat; cow; plasma; brain

REFERENCE
Bonate,P.L.; Davis,C.M.; Silverman,P.B.; Swann,A. Determination of cocaine in biological matrices using reversed phase HPLC: Application to plasma and brain tissue, *J.Liq.Chromatogr.*, **1995**, *18*, 3473–3494.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 244

CHROMATOGRAM
Retention time: 15.223

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Open capsules, weigh out amount equivalent to 5 mg clidinium bromide, add 15 mL water, sonicate for 10 min with gentle swirling. Make up to 25 mL with water, centrifuge, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 ODS-3
Mobile phase: MeCN:300 mM $(NH_4)H_2PO_4$ 32:68, adjust pH to 4.3 ± 0.1 with 10% phosphoric acid
Flow rate: 1

Injection volume: 10
Detector: UV 235

CHROMATOGRAM
Retention time: 7.1

OTHER SUBSTANCES
Simultaneous: clidinium bromide, impurities

KEY WORDS
capsules

REFERENCE
Yuen,S.M.; Lehr,G. Liquid chromatographic determination of clidinium bromide and clidinium bromide-chlordiazepoxide hydrochloride combinations in capsules, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 461–464.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Irradiate MeCN solutions with UV light, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:THF:60 mM pH 5.8 phosphate buffer 22:2:76
Column temperature: 20
Flow rate: 1
Injection volume: 20-100
Detector: UV 265

CHROMATOGRAM
Retention time: 10.25
Limit of detection: 0.5 ng

OTHER SUBSTANCES
Extracted: desmethylchlordiazepoxide, demoxepam, desmethyldiazepam, oxazepam

REFERENCE
Soentjens-Werts,V.; Dubois,J.G.; Atassi,G.; Hanocq,M. High-performance liquid chromatographic determination of chlordiazepoxide, its metabolites and oxaziridines generated after UV irradiation, *J.Chromatogr.A*, **1994**, *662*, 255–262.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:% for 2 min, to 0:100 over 15 min, maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: desalkylflurazepam, diazepam, flurazepam, norchlordiazepoxide, nordiazepam, oxazepam, prazepam

REFERENCE
Rainin Catalog 1991-2, p. 3.26.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 13.1

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: norchlordiazepoxide, oxazepam, nordiazepam, desalkylflurazepam, diazepam, flurazepam, prazepam

Also analyzed: amitriptyline, amphetamine, chlorpromazine, desipramine, desmethyldoxepin, diethylpropion, doxepin, ephedrine, fenfluramine, imipramine, mesoridazine, methamphetamine, nortriptyline, phentermine, phenylpropanolamine, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 240

CHROMATOGRAM

Retention time: 1.5
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, carbamazepine, chlorprothixene, clonazepam, caffeine, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions
Sample preparation: Dilute in MeOH to a concentration of 10-80 mg/mL, inject an aliquot

HPLC VARIABLES

Column: 150 × 3.9 5 μm Nova pak RP 18
Mobile phase: MeOH:water 50:50
Column temperature: 50
Flow rate: 0.82
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Simultaneous: bromazepam, nitrazepam, flunitrazepam, clobazam, lorazepam, oxazepam, tofisopam, chlorazepate, diazepam

KEY WORDS

conditions are optimized

REFERENCE

Guillaume,Y.; Guinchard,C. Study and optimization of column efficiency in HPLC: Comparison of two methods for separating ten benzodiazepines, *J.Liq.Chromatogr.*, **1994**, *17*, 1443–1459.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline,

tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova pack C18
Mobile phase: MeOH:water 52:48
Column temperature: 48
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: bromazepam, clobazam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

REFERENCE

Guillaume,Y.; Guinchard,C. Thermodynamic behavior of mixed benzodiazepines by a new liquid chromatographic method, *Chromatographia*, **1995**, *40*, 193–196.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova pak C18
Mobile phase: MeCN:water 57:43
Column temperature: 44
Flow rate: 1.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: bromazepam, clobazam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

REFERENCE

Guillaume,Y.; Guinchard,C. Marked difference between acetonitrile/water and methanol/water mobile phase systems on the thermodynamic behavior of benzodiazepines in reversed phase liquid chromatography, *Chromatographia*, **1995**, *41*, 84–87.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.85 (A), 5.26 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: MeOH:buffer 85:15 (Buffer was 90.7 mL 66.7 mM Na_2HPO_4 and 9.3 mL 66.7 mM KH_2PO_4 made up to 1 L with water, pH 7.6.)
Flow rate: 5 (sic)
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 6.06
Limit of detection: 100 nM

OTHER SUBSTANCES
Simultaneous: diazepam, flurazepam, nitrazepam

KEY WORDS
comparison with capillary electrophoresis; capillary GC; and polarography

REFERENCE
McGrath,G.; McClean,S.; O'Kane,E.; Smyth,W.F.; Tagliaro,F. Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay, *J.Chromatogr.A*, **1996**, *735*, 237–247.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 3 mL 5 M NaOH, vortex 30 s, add 12 mL diethyl ether, rotate for 5 min, centrifuge at 2500 rpm for 5 min. Remove the ether layer and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C18
Mobile phase: MeOH:water 50:50 containing 7 mL/L butylamine, adjusted to pH 3.2 with sulfuric acid
Flow rate: 1.8
Injection volume: 50
Detector: E, Bioanalytical Systems Model LC4B, dual glassy carbon working electrode cell half operated in the parallel mode + 1.0 V and +0.9 V, stainless steel auxiliary electrode cell half, Ag/AgCl reference electrode. The detector was preceded by a Photronix Model 816 UV irradiator which irradiated the mobile phase in a 9.144 m length of 0.5 mm i.d. × 1.6 mm o.d. Teflon tubing in a three-dimensional figure eight configuration. The irradiation apparatus was maintained at 0-5° using an ice bath.

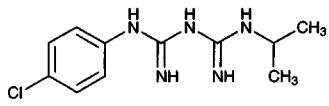
CHROMATOGRAM
Retention time: 6
Limit of detection: 2 ppb

OTHER SUBSTANCES
Simultaneous: methylphenidate, phenobarbital, nitrazepam
Interfering: cocaine

KEY WORDS
post-column photochemical derivatization

REFERENCE
Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, *23*, 499–508.

Chlorguanide



Molecular formula: $C_{11}H_{16}ClN_5$
Molecular weight: 253.73
CAS Registry No.: 500-92-5, 637-32-1 (HCl)
Merck Index: 2138
Lednicer No.: 1 115

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 6.55

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.61

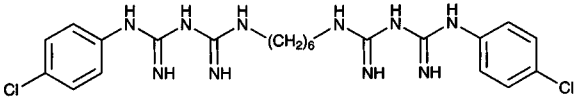
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Chlorhexidine



Molecular formula: $C_{22}H_{30}Cl_2N_{10}$

Molecular weight: 505.45

CAS Registry No.: 55-56-1, 3697-42-5 (dihydrochloride), 18472-51-0 (gluconate), 77146-42-0 (phosphanilate)

Merck Index: 2140

SAMPLE

Matrix: blood, urine

Sample preparation: 500 µL Serum or urine + 50 µL 50 µg/mL chlorpheniramine in water + 100 µL 2 M NaOH + 2.5 mL chloroform:isopropanol 95:5, shake vigorously for 20 min, centrifuge at 700 g for 5 min. Remove the organic layer and add it to 100 µL 50 mM sulfuric acid, mix vigorously on a shaker for 10 min, inject a 20 µL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:water 70:30 containing 5 mM sodium heptanesulfonate

Flow rate: 1.2

Injection volume: 20

Detector: UV 260

CHROMATOGRAM

Retention time: 8.0

Internal standard: chlorpheniramine (4.9)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: p-chloroaniline

Noninterfering: benzyl alcohol, benzoic acid, phosphanilic acid

KEY WORDS

serum

REFERENCE

Brougham,L.R.; Cheng,H.; Pittman,K.A. Sensitive high-performance liquid chromatographic method for the determination of chlorhexidine in human serum and urine, *J.Chromatogr.*, **1986**, *383*, 365–373.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.532

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 10 mg/mL solution in MeCN:100 mM pH 5.0 ammonium acetate 20:80.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Nucleosil C18 (A) or 250 × 4.6 5 μm Zorbax RX-C8 (B)
Mobile phase: A was MeCN. B was 100 mM pH 5.0 ammonium acetate. Gradient. A:B from 20:80 to 25:75 over 25 min, to 40:60 over 25 min, to 50:50 over 40 min, return to initial conditions, re-equilibrate for 10 min.
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 45-50 (A) or 45 (B)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison of columns

REFERENCE
Doub,W.H.; Ruhl,D.D.; Hart,B.; Mehelic,P.R.; Revelle,L.K. Gradient liquid chromatographic method for the determination of chlorhexidine and its degradation products in bulk material, *J.AOAC Int.*, **1996**, *79*, 636–639.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1.25 g ground pastilles, add 50 mL mobile phase, stir mechanically until dissolved, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:100 mM pH 5 potassium phosphate buffer containing 5.9 g/L NaCl and 30 mM tetrabutylammonium hydrogen sulfate
Flow rate: 2
Injection volume: 50
Detector: UV 294

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: degradation products, n-butyl p-aminobenzoic acid, p-chloroaniline, tetracaine

KEY WORDS
pastilles

REFERENCE
Bauer,M.; Degude,C.; Mailhe,L. Simultaneous determination of chlorhexidine, tetracaine and their degradation products by ion-pair liquid chromatography, *J.Chromatogr.*, **1984**, *315*, 457–464.

SAMPLE
Matrix: saliva
Sample preparation: Centrifuge saliva at 200 g, remove a 200 μL aliquot, add 200 μL 5 M KOH, vortex for 30 s, add 2 mL dichloromethane, shake vigorously on a horizontal shaker for 10 min, centrifuge at 200 g for 10 min. Remove the organic phase and evaporate it under a stream of nitrogen, reconstitute with 100 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:50 mM sodium acetate + 5 mM heptanesulfonic acid 40:60 adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 60
Detector: UV 260

CHROMATOGRAM
Retention time: 6.1
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: erythromycin, lidocaine, tetracycline

REFERENCE
Lam,Y.W.F.; Chan,D.C.N.; Rodriguez,S.Y.; Lintakoon,J.H.; Lam,T.-H. Sensitive high-performance liquid chromatographic assay for the determination of chlorhexidine in saliva, *J.Chromatogr.*, **1993**, *612*, 166–171.

SAMPLE
Matrix: saliva
Sample preparation: Collect sample on Periopaper strip (filter paper), add paper to 100 μL MeCN:water:glacial acetic acid 55:44.8:0.2 containing 7 mM sodium lauryl sulfate and 2 μg/mL benzethonium, vortex for 1 min, sonicate for 20 min, vortex, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm C18 ODS-B Exsil (HiChrome)

Mobile phase: MeCN:0.2% acetic acid 55:45 containing 5 mM sodium lauryl sulfate
Flow rate: 0.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: benzethonium (7)
Limit of detection: 2000 ng/mL

KEY WORDS
narrow-bore

REFERENCE
Medlicott,N.J.; Ferry,D.G.; Tucker,I.G.; Rathbone,M.J.; Holborow,D.W.; Jones,D.S. High performance liquid chromatographic (HPLC) assay for the determination of chlorhexidine in saliva film, *J.Liq.Chromatogr.*, **1994**, *17*, 1605–1620.

SAMPLE
Matrix: saliva
Sample preparation: 200 μL Saliva + 400 μL 4.5 M NaOH + 400 μL MeCN, vortex for 1 min, centrifuge for 1 min at 14000 g. Remove 200 μL of the organic phase and add it to 370 μL buffer, mix, inject a 20 μL aliquot. (Buffer was 100 mM Na_2HPO_4 containing 5 mM 1-heptanesulfonic acid and 50 mM triethylamine, pH adjusted to 2.5 with phosphoric acid.)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 100 mM Na_2HPO_4 containing 5 mM 1-heptanesulfonic acid and 50 mM triethylamine, pH adjusted to 2.5 with phosphoric acid.)
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 2.10
Limit of quantitation: 500 ng/mL

KEY WORDS
pharmacokinetics

REFERENCE
Pesonen,T.; Holmalahti,J.; Pohjola,J. Determination of chlorhexidine in saliva using high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 222–225.

SAMPLE
Matrix: solutions
Sample preparation: 9.5 mL Contact lens solution + 0.5 mL 3 mg/mL methylparaben, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 7 μm Nucleosil C18 pre-column
Column: 7 μm Nucleosil C18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 3.5 with phosphoric acid 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 14.9 (chlorhexidine gluconate)
Internal standard: methyl paraben (7.8)
Limit of detection: 200 ng

OTHER SUBSTANCES
Simultaneous: thimerosal, thiosalicylic acid

KEY WORDS
stability-indicating; contact lens solutions

REFERENCE
Hu,O.Y.-P.; Wang,S.-Y.; Fang,Y.-J.; Chen,Y.-H.; King,M.-L. Simultaneous determination of thimerosal and chlorhexidine in solutions for soft contact lenses and its applications in stability studies, *J.Chromatogr.*, **1990**, *523*, 321–326.

SAMPLE
Matrix: urine
Sample preparation: Direct injection of a 20 μL aliquot.

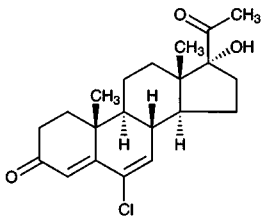
HPLC VARIABLES
Column: 250 × 5 10 μm Partisil silica
Mobile phase: MeOH:2 M ammonia:1 M ammonium nitrate 90:5:5
Flow rate: 3
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: 1000 ng/mL

KEY WORDS
normal phase; use a 50 mm long column of 27-44 μm silica between pump and injection valve to saturate mobile phase with silica

REFERENCE
Wainwright,P.; Cooke,M. Direct determination of chlorhexidine in urine by high-performance liquid chromatography, *Analyst*, **1986**, *111*, 1343–1344.

Chlormadinone



Molecular formula: $C_{21}H_{27}ClO_3$
Molecular weight: 362.90
CAS Registry No.: 1961-77-9, 302-22-7 (acetate)
Merck Index: 2152
Lednicer No.: 1 181

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 283.1

CHROMATOGRAM

Retention time: 24.11

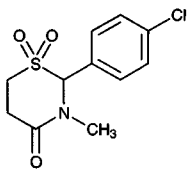
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Chlormezanone



Molecular formula: $C_{11}H_{12}ClNO_3S$
Molecular weight: 273.74
CAS Registry No.: 80-77-3
Merck Index: 2155

SAMPLE

Matrix: blood
Sample preparation: 1 mL plasma + 100 mg ammonium sulfate + 2 mL ethyl acetate, vortex for 2 min, sonicate for 3 min, centrifuge at 6000 rpm for 10 min. Remove 1 mL of the supernatant and evaporate it to dryness under vacuum, reconstitute the residue in 500 μL mobile phase, inject a 20-40 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4 7.5 μm Nucleosil C18
Column: 120 × 4 7.5 μm Nucleosil C18
Mobile phase: MeOH:water:85% orthophosphoric acid 50:50:1
Flow rate: 1
Injection volume: 20-40
Detector: UV 228

CHROMATOGRAM

Retention time: 3.37
Limit of detection: 100 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen
Noninterfering: codeine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Ali,S.L.; Blume,H. Determination of chlormezanone in human plasma after administration of chlormezanone formulations, *Arzneimittelforschung*, **1987**, *37*, 1396–1399.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 300 μL MeCN, mix, allow to stand for 10 min, mix, centrifuge for 5 min, inject an aliquot of the supernatant

HPLC VARIABLES

Guard column: 20 × 4 5 μm Nucleosil C18
Column: 120 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:water:85% orthophosphoric acid 35:65:0.1
Flow rate: 1.5
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Retention time: 4.12
Limit of detection: 50 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Koppel,C.; Kristinsson,J.; Wagemann,A.; Tenczer,J.; Martens,F. Chlormezanone plasma and blood levels in patients after single and repeated oral doses and after suicidal drug overdose, *Eur.J.Drug Metab.Pharmacokinet.*, **1991**, *16*, 43–47.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 223

CHROMATOGRAM
Retention time: 3.40
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine;

aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.493

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.03 (A), 5.33 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

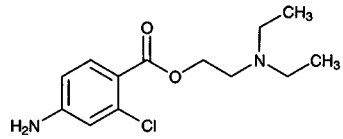
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Chloroprocaine



Molecular formula: $C_{13}H_{19}ClN_2O_2$
Molecular weight: 270.76
CAS Registry No.: 133-16-4, 3858-89-7 (HCl)
Merck Index: 2210
Lednicer No.: 1 11

SAMPLE

Matrix: bulk, formulations
Sample preparation: 100 mg Bulk drug or formulation containing 100-120 mg drug + 10 mL 1 (bulk) or 4 (formulations) mg/mL benzoic acid in MeOH:water 50:50 + 20 mL 5 mg/mL p-nitroacetophenone in MeOH, make up to 100 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:glacial acetic acid 20:5:74:1 containing 0.05-0.08% sodium 1-heptanesulfonate, pH 3.1
Flow rate: 2
Injection volume: 5
Detector: UV 278

CHROMATOGRAM

Retention time: 9
Internal standard: benzoic acid (6), p-nitroacetophenone (12)

OTHER SUBSTANCES

Simultaneous: 4-amino-2-chlorobenzoic acid, impurities

REFERENCE

Menon,G.; Norris,B.; Webster,J. Simultaneous determination of chloroprocaine hydrochloride and its degradation product 4-amino-2-chlorobenzoic acid in bulk drug and injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 251–253.

SAMPLE

Matrix: perfusate
Sample preparation: Adjust pH of 5-10 mL perfusate to 5 with 180 μL 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water containing 30 μL/L triethylamine, adjusted to pH 2.3 with phosphoric acid 20:80
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Retention time: 9.2

OTHER SUBSTANCES

Extracted: 2-chloro-4-aminobenzoic acid, 4-acetamidohippuric acid

KEY WORDS
rabbit; chinchilla; pharmacokinetics

REFERENCE
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, *22*, 1357–1366.

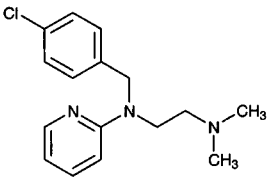
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 0.92

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Chloropyramine



Molecular formula: $C_{16}H_{20}ClN_3$
Molecular weight: 289.81
CAS Registry No.: 59-32-5, 6170-42-9 (HCl)
Merck Index: 2214
Lednicer No.: 1 402

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 21.38

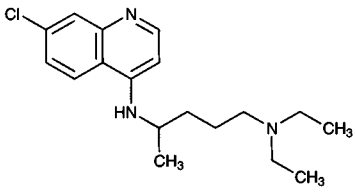
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Chloroquine



Molecular formula: $C_{18}H_{26}ClN_3$
Molecular weight: 319.88
CAS Registry No.: 54-05-7, 50-63-5 (phosphate), 3545-67-3 (HCl)
Merck Index: 2215
Lednicer No.: 1 341

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 7.5 I.D. Progel-TSK Heparin-5PW affinity column (Supelco)
Mobile phase: 20 mM pH 6.0 sodium phosphate buffer
Column temperature: 19
Flow rate: 0.8
Detector: UV 214

CHROMATOGRAM
Retention time: 37 (+), 44 (-)

KEY WORDS
chiral

REFERENCE
Stalcup,A.M.; Gahm,K.H.; Baldueza,M. Chiral separation of chloroquine using heparin as a chiral selector in high-performance liquid chromatography, *Anal.Chem.*, **1996**, *68*, 2248–2250.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M NaOH + 30 mL n-heptane, shake for 30 min. Remove 25 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 µL MeOH:100 mM phosphoric acid 50:50, inject a 10 µL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:20 mM pH 3.4 1-heptanesulfonic acid 34:66 (Aqueous solution was 40 mL Pic B-7 (Waters) + 460 mL water, pH 3.4.)
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 9
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Brown,N.D.; Poon,B.T.; Chulay,J.D. Determination of chloroquine and its de-ethylated metabolites in human plasma by ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *229*, 248–254.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood or plasma + 1 mL water + 50 μL 10 μg/mL IS in MeOH + 1 mL 60% aqueous KOH, vortex for 1 min, heat for 3 min on a boiling water bath, pass onto a 3 mL Extrelut cartridge. Elute with diethyl ether:dichloromethane 70:30, evaporate eluate to dryness under a stream of air at 40°, vortex in 100 μL initial mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was KH_2PO_4: 1 N phosphoric acid 999:1. B was MeCN. A:B 65:35 for 12 min at 1 mL/min and UV 343 nm then 55:45 at 2 mL/min and UV 242 nm (step gradient).
Flow rate: 1-2
Injection volume: 40
Detector: UV 343, UV 242

CHROMATOGRAM
Retention time: 5.35
Internal standard: papaverine hydrochloride (10.7 min)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, diazepam, monodesethylchloroquine, nordiazepam
Simultaneous: epinephrine, sulfadoxine, quinine, pyrimethamine, mefloquine

KEY WORDS
whole blood; plasma

REFERENCE
Estadieu,M.; Durand,A.; Viala,A.; Rop,P.P.; Fornaris,M.; Quicke,J. A rapid HPLC procedure for the simultaneous determination of chloroquine, monodesethylchloroquine, diazepam, and nordiazepam in blood, *J.Anal.Toxicol.*, **1989**, *13*, 89–93.

SAMPLE
Matrix: blood
Sample preparation: Serum. 200 μL Serum + 50 μL 1 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Whole blood. 100 μL Whole blood + 500 μL water + 50 μL 1 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Dried blood. Spread 100 μL whole blood on a 70 × 30 mm piece of filter paper, allow to dry, cut paper into 10 × 5 mm strips, add 100 μL 1 μg/mL IS in water, add 1.5 mL 0.5 M NaOH, vortex for 30 s, let stand for 30 min at room temperature, add 300 μL MTBE, vortex for 30 s, centrifuge at 2000 g for 5 min, inject a 100 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 5 5 μm Spherisorb S5SCX sulfophenylpropyl-modified silica
Mobile phase: MeOH:water 98.5:1.5 containing 9.41 g/L ammonium perchlorate, adjust apparent pH to 8.0 with 220 mL/L 50 mM NaOH in MeOH
Flow rate: 1.5
Injection volume: 100

Detector: F ex 215 em no filter

CHROMATOGRAM

Retention time: 14
Internal standard: 6,8-dichloro-4-(1-methyl-4-diethylaminobutylamino)quinoline (5)
Limit of quantitation: 5 ng/mL (serum), 10 ng/mL (whole blood, dried blood)

OTHER SUBSTANCES

Extracted: hydroxychloroquine, quinine, metabolites
Simultaneous: acebutolol, N-acetylprocainamide, atenolol, butriptyline, chlorpromazine, desipramine, flecainide, fluoxetine, imipramine, labetalol, maprotiline, mepacrine, metoprolol, mexiletine, norbutriptyline, normaprotiline, procainamide, propranolol, sotalol
Noninterfering: amitriptyline, amodiaquin, carbamazepine, clomipramine, dapsone, diazepam, dothiepin, doxepin, fluvoxamine, lorazepam, mefloquine, nitrazepam, norclomipramine, nordiazepam, nordothiepin, nordoxepin, nortriptyline, primaquine, proguanil, pyrimethamine

KEY WORDS

serum; whole blood; dried blood

REFERENCE

Croes,K.; McCarthy,P.T.; Flanagan,R.J. Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood, *J.Anal.Toxicol.*, **1994**, *18*, 255–260.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 4.72
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam;

clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.

Flow rate: 1

Injection volume: 20

Detector: UV 245, 256, 343

CHROMATOGRAM

Retention time: 13.20

Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)

Limit of detection: 2 ng/mL (343 nm)

OTHER SUBSTANCES
Extracted: betamethasone, corticosterone, cortisone, dexamethasone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: blood
Sample preparation: Dilute urine 10-fold with water. 1 mL Plasma or urine + 4 mL diethyl ether + 10 (plasma) or 20 (urine) μL 5 μg/mL papaverine + 1 mL 2 M NaOH, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, vortex for 1 min, inject a 10 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Bondapak
Mobile phase: MeCN:MeOH:200 mM NaH_2PO_4 5:30:65 containing 10 mL/L perchloric acid, pH 3.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: papaverine (6)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: halofantrine, mefloquine, pyrimethamine, sulfadoxine, tetracycline

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Walker,O.; Ademowo,O.G. A rapid, cost-effective liquid chromatographic method for the determination of chloroquine and desethylchloroquine in biological fluids, *Ther.Drug Monit.*, **1996**, *18*, 92–96.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Urine or 1 mL plasma + 100 ng IS + 1 mL MeCN, centrifuge. Remove supernatant and add it to 1 mL buffer, add 6 mL dichloromethane:petroleum ether:isopropanol 45:45:10, rotate for 10 min. Remove the upper organic layer and evaporate it under nitrogen at 60°. Dissolve residue in 100 μL mobile phase, inject. (Buffer contained 80 g $NaHCO_3$ and 30 g K_2CO_3 per liter, pH 9.5.)

HPLC VARIABLES
Column: 466 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:MeOH:45 mM pH 4.5 KH_2PO_4 40:3:57, containing 40 g/L $NaClO_4$ and 40 g/L trimethylammonium chloride

Flow rate: 1
Injection volume: 100
Detector: UV 338

CHROMATOGRAM
Retention time: 6.5
Internal standard: 4-(3-dimethylaminopropyl)-4-chloroquinoline (5)
Limit of quantitation: < 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metronidazole

KEY WORDS
plasma

REFERENCE
Okonkwo,P.O.; Eta,E.I. Simultaneous determination of chloroquine and metronidazole in human biological fluid by high-pressure liquid chromatography, *Life Sci.*, **1988**, *42*, 539–545.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 221.6

CHROMATOGRAM
Retention time: 5.442

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, erythrocytes, urine

Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL buffer. Hemolyze erythrocytes in water 1:3. Dilute urine with water 1:99. 1 mL Plasma, hemolyzed erythrocytes, or diluted urine + 100 μL 5 μg/mL hydroxychloroquine sulfate in MeOH:water 50:50, mix, add to the SPE cartridge, wash with 4 mL buffer, wash with 2 mL MeOH:buffer 50:50, elute with 3 mL MeOH:ammonia 99:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in the initial mobile phase, vortex, inject a 50 μL aliquot. (Prepare buffer by mixing equal volumes of 100 mM ammonium formate and 100 mM ammonia solution, pH 9.2.)

HPLC VARIABLES

Guard column: 10 × 4 Inertsil
Column: 250 × 4 5 μm Inertsil
Mobile phase: Gradient. A was MeCN. B was MeOH:25% ammonia solution 92.5:7.5. A:B 78:22 for 3 min, then to 65:35 over 2 min (Waters curve no. 3), maintain at 65:35 for 20 min, return to 78:22 over 5 min (Waters curve no. 3).
Flow rate: 0.85
Injection volume: 50
Detector: F ex 325 em 375

CHROMATOGRAM

Retention time: 15.6
Internal standard: hydroxychloroquine sulfate (11.5)
Limit of detection: 4.7 ng/mL
Limit of quantitation: 5.6 ng/mL

OTHER SUBSTANCES

Extracted: quinine, monodesethylchloroquine, bidesethylchloroquine
Simultaneous: halofantrine, quinidine
Noninterfering: proguanil, cycloguanil, 4-chlorophenylbiguanide, amodiaquine, mefloquine, pyrimethamine, sulfadoxine, cinchonine, cinchonidine

KEY WORDS

plasma; SPE

REFERENCE

Chaulet,J.-F.; Robet,Y.; Prevosto,J.-M.; Soares,O.; Brazier,J.-L. Simultaneous determination of chloroquine and quinine in human biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 303–310.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with saline, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Lichrosorb 10 RP 8
Mobile phase: THF:triethylamine:water 4:0.75:96, adjusted to a pH of 2.3 with 2 M nitric acid
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5.2

KEY WORDS

injections; saline

REFERENCE

Martens,H.J.; de Goede,P.N.; van Loenen,A.C. Sorption of various drugs in polyvinyl chloride, glass, and polyethylene-lined infusion containers, *Am.J.Hosp.Pharm.*, **1990**, *47*, 369–373.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Mix 200 μL microsomal incubation with 200 μL ice-cold MeCN, vortex for 1 min, keep at 4° for 10-15 min. Centrifuge at 600 g at 4° for 10 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Spherisorb C1
Mobile phase: MeOH:water 70:30 containing 7 mM (0.1%) triethylamine
Flow rate: 1
Injection volume: 15
Detector: F ex 250 em 380

CHROMATOGRAM

Retention time: 12.8-13.1
Limit of detection: 1 nM
Limit of quantitation: 78 nM

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: coumarin, diethyldithiocarbamate, quinidine, ketoconazole, mephenytoin, α-naphthoflavone, sulfaphenazole, tolbutamide

KEY WORDS

liver

REFERENCE

Ducharme,J.; Farinotti,R. Rapid and simple method to determine chloroquine and its desethylated metabolites in human microsomes by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *698*, 243–250.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 0.99

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(4-tert-butylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: 30 (-), 34 (+)

KEY WORDS
chiral

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapy-

rilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 50:35:15 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 346

KEY WORDS

chiral; α = 1.22 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 5 Spherisorb S5SCX
Mobile phase: MeOH:water 98.5:1.5 containing 80 mM ammonium perchlorate, adjusted to pH 8.0 with 50 mM NaOH in MeOH
Flow rate: 1.5
Detector: F ex 215 no emission filter

CHROMATOGRAM

Retention time: 13.5
Internal standard: hydroxychloroquine (8)

OTHER SUBSTANCES
Simultaneous: hydroquinine, quinine

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 12.70 (A), 3.55 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 400 μL Urine + 400 μL 1 M NaOH + 30 mL n-heptane, shake for 30 min. Remove 25 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL MeOH:100 mM phosphoric acid 50:50, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 300 × 7.8 10 μm μBondapak C18

Mobile phase: MeCN:20 mM pH 3.4 1-heptanesulfonic acid 35:65 (Aqueous solution was 40 mL Pic B-7 (Waters) + 460 mL water, pH 3.4.)

Flow rate: 2

Injection volume: 30

Detector: UV 340

CHROMATOGRAM

Retention time: 18

Limit of detection: 2 ng

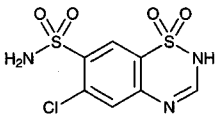
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Brown,N.D.; Poon,B.T.; Chulay,J.D. Chloroquine metabolism in man: urinary excretion of 7-chloro-4-hydroxyquinoline and 7-chloro-4-aminoquinoline metabolites, *J.Chromatogr.*, **1985**, *345*, 209–214.

Chlorothiazide



Molecular formula: $C_7H_6ClN_3O_4S_2$
Molecular weight: 295.73
CAS Registry No.: 58-94-6, 7085-44-1 (sodium salt)
Merck Index: 2221
Lednicer No.: 1 321

SAMPLE

Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 5.43
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 5.67
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 10 mL DMSO, shake vigorously for 5 min, make up to 100 mL with MeOH, mix, filter (paper), discard the first 5 mL filtrate. Dilute 10 mL of the filtrate to 100 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 75 × 3.9 Novapak silica
Mobile phase: MeOH:20 g/L sodium 1-pentanesulfonate in water 100:1
Flow rate: 1
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: reserpine (F ex 280 em 360)

KEY WORDS
tablets

REFERENCE
Cieri,U.R. Determination of reserpine and chlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorbance detectors in series, *J.AOAC Int.*, **1995**, *78*, 1384–1387.

SAMPLE
Matrix: solutions

Sample preparation: Dilute 75 mL 1.2 mg/mL chlorothiazide in MeOH with 75 mL 380 mg/L IS in 0.1% phosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: A 250 × 2 J sphere ODS-M80; B 150 × 4.6 5 μm Beckman Ultrasphere C18
Mobile phase: A Gradient. MeCN:0.1% formic acid from 0:100 to 30:70 over 20 min; B Gradient. MeCN:0.1% phosphoric acid from 0:100 to 30:70 over 12 min.
Flow rate: A 0.2; B 1
Detector: A MS, Finnigan Model TSQ-7000 triple-quadrupole, nebulizer nitrogen 260°; B UV 270

CHROMATOGRAM
Retention time: 20.5
Internal standard: ethylparaben

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
photolysis

REFERENCE
Revelle,L.K.; Musser,S.M.; Rowe,B.J.; Feldman,I.C. Identification of chlorothiazide and hydrochlorothiazide UV-A photolytic decomposition products, *J.Pharm.Sci.*, **1997**, *86*, 631–634.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 2 C18 glass lined (Whatman)
Mobile phase: MeCN:water 60:40
Flow rate: 0.04
Injection volume: 0.5
Detector: UV 254 or MS, Hewlett Packard 5985, home-made interface (details in paper)

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide, trichlormethiazide

KEY WORDS
microbore

REFERENCE
Eckers,C.; Skrabalak,D.S.; Henion,J. On-line direct liquid introduction interface for micro-liquid chromatography/mass spectrometry: application to drug analysis, *Clin.Chem.*, **1982**, *28*, 1882–1886.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5

Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 0.31

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Irradiate an ethanolic solution at 313 nm, inject a 3 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeOH:water:acetic acid 10:88:2, pH 2.7
Flow rate: 1
Injection volume: 3
Detector: UV 265

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Ulvi,V.; Tammilehto,S. High-performance liquid chromatographic method for studies on the photodecomposition kinetics of chlorothiazide, *J.Chromatogr.*, **1990**, *507*, 151–156.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal,

digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 µL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 µm LiCHrosorb RP-18

Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min

Column temperature: 50

Flow rate: 1.5

Injection volume: 5

Detector: UV 271

CHROMATOGRAM

Retention time: 1.8

Limit of quantitation: 1500 ng/mL

OTHER SUBSTANCES

Extracted: hydrochlorothiazide, quinethazone, chlorthalidone, methyclothiazide, clopamide, furosemide, metolazone, mefruside, bendroflumethiazide, cyclopenthiazide, bumetanide

Simultaneous: indapamide, clorexolone, ethacrynic acid

Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE

Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 µL 50 µg/mL β-hydroxyethyltheophylline in MeOH, inject 5 µL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 5.37 (A), 6.43 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, hydrochlorothiazide, quinethazone, triamterene, flumethiazide, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 10.8

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

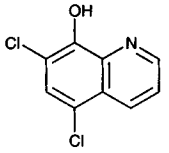
KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

Chloroxine



Molecular formula: $C_9H_5Cl_2NO$
Molecular weight: 214.05
CAS Registry No.: 773-76-2
Merck Index: 2227

SAMPLE

Matrix: formulations
Sample preparation: Weigh out shampoo containing 30 mg of chloroxine, add 40-50 mL MeOH, dissolve with heating, make up to 100 mL with MeOH. Remove a 2 mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 3 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeCN:MeOH:water 30:20:50 containing 1 mM $NiCl_2$
Flow rate: 1.2
Detector: UV 273

CHROMATOGRAM

Retention time: 6.5
Internal standard: diphenylamine (11)

OTHER SUBSTANCES

Simultaneous: iodochlorhydroxyquin, iodoquinol

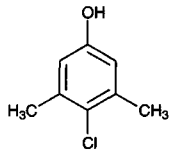
KEY WORDS

shampoo; separated as Ni chelates

REFERENCE

Wojtowicz,E.J. Reverse-phase high-performance liquid chromatographic determination of halogenated 8-hydroxyquinoline compounds in pharmaceuticals and bulk drugs, *J.Pharm.Sci.*, **1984**, *73*, 1430–1433.

Chloroxylenol



Molecular formula: C_8H_9ClO
Molecular weight: 156.61
CAS Registry No.: 88-04-0
Merck Index: 2228

SAMPLE

Matrix: formulations
Sample preparation: Sonicate an amount of cream or ointment containing 230 μg chloroxylenol with 10 mL DMSO at 40° for 10 min, decant. Sonicate a 200 μL aliquot of the clear solution with 30 μL 60 mM potassium carbonate solution for 10 min. Add 400 μL 2.5 mg/mL 2-chloro-6,7-dimethoxy-3-quinoline carboxaldehyde solution in DMSO. Stir the reaction mixture at 110° for 50 min. Add 60 μL 2 M acetic acid and 800 μL IS in the mobile phase. Sonicate the mixture for 1 min. Inject a 50 μL aliquot. (Details for the preparation of 2-chloro-6,7-dimethoxy-3-quinoline carboxaldehyde are given in Tetrahedron Letters 1978, 23, 2045.)

HPLC VARIABLES

Column: 250 × 4.6 Hypersil 5ODS
Mobile phase: MeCN:THF:50 mM pH 3.0 triethyl ammonium phosphate buffer 49.4:2.6:48
Column temperature: 35
Flow rate: 1.3
Injection volume: 50
Detector: F ex 360 em 500

CHROMATOGRAM

Retention time: 27.8 (4-chloro-3,5-xylenol)
Internal standard: 4-chloro-3-cresol (19.5)
Limit of detection: 1 pmol

KEY WORDS

derivatization; cream; ointment

REFERENCE

Gatti,R.; Roveri,P.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of chlorocresol and chloroxylenol in pharmaceuticals, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 405–412.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 21.4

OTHER SUBSTANCES

Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS

details for purification of triethylamine in paper

REFERENCE

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

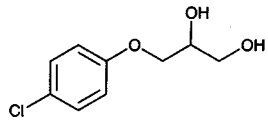
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargy-

line, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Chlorphenesin



Molecular formula: $C_9H_{11}ClO_3$
Molecular weight: 202.64
CAS Registry No.: 104-29-0
Merck Index: 2230
Lednicer No.: 1 118

SAMPLE

Matrix: reaction mixtures
Sample preparation: 5 mL Reaction mixture + 1 mL 5 M HCl, add 2 g naCl, add 2 mL dichloromethane:n-butanol 50:50, shake for 5 min, centrifuge at 3000 rpm for 10 min. Remove the upper organic layer and extract the lower aqueous layer with 2 mL and with 1 mL of dichloromethane:n-butanol 50:50. Combine the organic layers and make up to 5 mL with dichloromethane:n-butanol 50:50, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 500 × 4 5 µm Lichrosorb SI 100
Mobile phase: Water-saturated dichloromethane:dichloromethane:n-butanol:n-hexane: MeOH 38.5:38.5:10:10:3
Flow rate: 1.3
Injection volume: 10
Detector: UV 233

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: chlorphenesin carbamate

KEY WORDS

normal phase

REFERENCE

Hara,M.; Hayashi,H.; Yoshida,T.; Murayama,H. Studies on the kinetics and mechanism of drug degradation. I. Kinetics and mechanism of degradation of chlorphenesin carbamate in strongly alkaline aqueous solutions, *Chem.Pharm.Bull.(Tokyo)*, **1986**, *34*, 1764–1769.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 3 5 µm Lichrospher 60 RP-Select-B
Mobile phase: B MeOH:30 mM ammonium formate 40:60
Flow rate: 0.5
Detector: UV 230

CHROMATOGRAM

Retention time: 7.2

OTHER SUBSTANCES

Simultaneous: cloxyquin, naftifine, sulbentine, tolnaftate, degradation products

REFERENCE

Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 60 RP-Select-B
Mobile phase: MeOH:20 mM ammonium formate buffer 40:60
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Retention time: 4.69

OTHER SUBSTANCES

Simultaneous: cloxyquin, naftifine, sulbentine, tolnaftate, degradation products

REFERENCE

Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

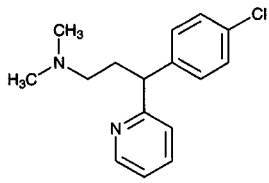
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone,

hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Chlorpheniramine



Molecular formula: $C_{16}H_{19}ClN_2$

Molecular weight: 274.79

CAS Registry No.: 132-22-9, 113-92-8 (maleate), 2438-32-6 (maleate), 25523-97-1 (d-form), 2438-32-6 (d-form maleate)

Merck Index: 2232

Lednicer No.: 1 77

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 5.60

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine;

aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: Plasma. Centrifuge blood at 7000 rpm, decant 100 μL plasma. Mix 100 μL plasma with 200 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25 μL water to 25 μL CSF, mix with 50 μL acetone, centrifuge at 7000 rpm for 5 min, decant the supernatant, evaporate under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: μBondapak C18

Mobile phase: MeOH:water 58.5:41.5 containing 20 mM butylamine

Column temperature: 60

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Chou,K.-J.; Donovan,M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm.Drug Dispos.*, **1997**, *18*, 335–346.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)

Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18

Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5

Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 8.86
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 100 μL 2 M NaOH + 2.5 mL chloroform: isopropanol 95:5, shake vigorously for 20 min, centrifuge at 700 g for 5 min. Remove the organic layer and add it to 100 μL 50 mM sulfuric acid, mix vigorously on a shaker for 10 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 70:30 containing 5 mM sodium heptanesulfonate
Flow rate: 1.2
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 4.9
Internal standard: chlorpheniramine

OTHER SUBSTANCES
Simultaneous: chlorhexidine, p-chloroaniline
Noninterfering: benzyl alcohol, benzoic acid, phosphanilic acid

KEY WORDS
serum; chlorpheniramine is IS

REFERENCE
Brougham,L.R.; Cheng,H.; Pittman,K.A. Sensitive high-performance liquid chromatographic method for the determination of chlorhexidine in human serum and urine, *J.Chromatogr.*, **1986**, *383*, 365–373.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.925

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell incubations
Sample preparation: 40 mL Cell incubation + 50 mL MeOH, shake vigorously for 1 min, centrifuge at 2000 rpm for 10 min, wash the pellet twice with 50 mL portions of MeOH. Combine the supernatants and add 100 mL water, extract three times with 150 mL portions of dichloromethane. Filter the extracts through anhydrous sodium sulfate, evaporate the filtrate to dryness under reduced pressure at 40°, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano-propyl (Beckman)
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM triethylamine, pH 7.0.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.4

OTHER SUBSTANCES
Extracted: metabolites
Interfering: brompheniramine, pheniramine

KEY WORDS
also semipreparative details

REFERENCE
Hansen,E.B.,Jr.; Cho,B.P.; Korfmacher,W.A.; Cerniglia,C.E. Fungal transformations of antihistamines: metabolism of brompheniramine, chlorpheniramine, and pheniramine to *N*-oxide and *N*-demethylated metabolites by the fungus *Cunninghamella elegans*, *Xenobiotica*, **1995**, *25*, 1081–1092.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 7.45

OTHER SUBSTANCES
Simultaneous: dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, pyridoxine, tetrahydrozoline, vitamin B12
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a powder, completely dissolve in 100 mL water, vortex mechanically for 5 min, centrifuge an aliquot at 3000 rpm for 5 min. Remove a 300 μL aliquot of the supernatant, add 20 μL 100 μg/mL IS in MeOH, dilute to 1 mL with MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 10 μm μBondapak C18
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM ammonium acetate adjusted to pH 3.5 with glacial acetic acid.)
Flow rate: 1.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5.42
Internal standard: propylparaben (8.45)
Limit of quantitation: 500 ng/mL

KEY WORDS
tablets

REFERENCE
Al-Deeb,O.A.; Foda,N.H.; El Shafie,F.; Al-Afifi,A. High performance liquid chromatographic assay of chlorpheniramine maleate in tablet formulations, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2221–2231.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Also analyzed: cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, dissolve in 100 mL 100 mM pH 5.0 acetate buffer, let sit for 1 h with occasional mixing, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax SCX
Mobile phase: MeCN:30 mM KH_2PO_4 50:50
Flow rate: 2
Injection volume: 50
Detector: UV 263

CHROMATOGRAM
Retention time: 16.0

OTHER SUBSTANCES
Simultaneous: pseudoephedrine, dextromethorphan

KEY WORDS
tablets

REFERENCE
Murtha,J.L.; Julian,T.N.; Radebaugh,G.W. Simultaneous determination of pseudoephedrine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide by second-derivative photodiode array spectroscopy, *J.Pharm.Sci.*, **1988**, *77*, 715–718.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add N,N-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Asahipak ODP-50 C18
Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 27:73
Flow rate: 0.8
Injection volume: 20-100
Detector: Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 7
Internal standard: N,N-dimethylbenzylamine
Limit of detection: 140 ng/mL

OTHER SUBSTANCES
Simultaneous: brompheniramine, diphenhydramine, pyrilamine, pheniramine

KEY WORDS
tablets

REFERENCE
Holeman,J.A.; Danielson,N.D. Liquid chromatography of antihistamines using post-column tris(2,2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, *679*, 277–284.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder half a tablet, add 9 mL mobile phase, sonicate for 20 min, make up to 10 mL with mobile phase, filter (Whatman type 40 and 0.2 μm Millipore), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 CN
Mobile phase: MeCN:THF:buffer 7:6:87 (Buffer was 0.8% acetic acid containing 5 mM sodium hexanesulfonate, 10 mM di-n-butylamine, and 0.12% phosphoric acid, pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 7.8

Limit of detection: 2.9 μg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen (UV 310), caffeine (UV 298), guaifenesin (glycerylguaicolate) (UV 284), phenylpropanolamine (UV 260)

KEY WORDS
tablets

REFERENCE
Indrayanto,G.; Sunarto,A.; Adriani,Y. Simultaneous assay of phenylpropanolamine hydrochloride, caffeine, paracetamol, glycerylguaiacolate and chlorpheniramine in Silabat™ tablet using HPLC with diode array detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1555–1559.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 21.2

OTHER SUBSTANCES
Simultaneous: bromhexine, codeine, dextromethorphan, diphenhydramine, ephedrine, papaverine, phenylephrine

KEY WORDS
syrup; indirect conductometric detection; presence of compound causes a decrease in mobile phase conductivity

REFERENCE
Lau,O.-W.; Mok,C.-S. High-performance liquid chromatographic determination of active ingredients in cough-cold syrups with indirect conductometric detection, *J.Chromatogr.A*, **1995**, *693*, 45–54.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 500 mg Bond Elut SCX strong cation-exchange SPE cartridge with 6 mL MeOH and 3 mL 10 mM pH 4.5 phosphate buffer. Weigh out powdered tablet containing 0.45 mg chlorpheniramine, add 25 mL MeCN:10 mM pH 4.5 phosphate buffer 25:75, sonicate for 10 min, dilute to 50 mL with 10 mM pH 4.5 phosphate buffer, centrifuge, add a 10 mL aliquot of the supernatant to the SPE cartridge, wash with four 3 mL portions of 10 mM pH 4.5 phosphate buffer, elute with 4 mL MeCN:100 mM pH 8.0 triethylamine phosphate 40:60, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:100 mM pH 3.0 triethylamine phosphate 5:95
Flow rate: 1
Injection volume: 20

Detector: UV 275 or UV 330 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID crocheted PTFE coil irradiated with an 8 W low-pressure mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Noninterfering: acetaminophen, caffeine

KEY WORDS
post-column reaction; post-column photochemical derivatization; SPE; tablets

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

SAMPLE
Matrix: iincubations
Sample preparation: Extract incubation mixture with four volumes of cold dichloromethane for 3 min, centrifuge at 1000 g for 5 min. Remove the organic layer and pass it through a nylon filter, evaporate the filtrate to dryness, reconstitute the residue in 500 μL MeOH, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm AXXIOM silica (Richard Scientific)
Mobile phase: MeCN:MeOH:60% aqueous perchloric acid 60:40:0.08
Flow rate: 1
Injection volume: 15
Detector: UV 260

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 5-20 ng/mL

OTHER SUBSTANCES
Extracted: brompheniramine N-oxide, chlorpheniramine N-oxide
Interfering: brompheniramine

KEY WORDS
ion-pair; desorption; chromatography; incubations

REFERENCE
Cashman,J.R.; Yang,Z.-C. Analysis of amine metabolites by high-performance liquid chromatography on silica gel with a non-aqueous ionic eluent, *J.Chromatogr.*, **1990**, *532*, 405–410.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 400 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 265

OTHER SUBSTANCES
Also analyzed: antipyrine

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine,

theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: thonzylamine, pheniramine, tripelennamine, brompheniramine, phenindamine, phenyltoxamine, clemizole

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 98:2
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: k' 1.75 (of first (-) enantiomer)

KEY WORDS

chiral; α 1.09

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 7.6 μg/mL solution, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: Supelguard LC-8-DB (Supelco)
Column: 50 × 4.6 Supelcosil LC-8-DB
Mobile phase: MeCN:buffer 10:90 containing 0.02% triethylamine (Buffer was KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)
Column temperature: 35
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: methscopolamine, phenylpropanolamine, pseudoephedrine, triprolidine

REFERENCE
Supelco Catalog, **1992,** p. 179.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: chlorcyclizine, clonidine, diphenhydramine, promethazine, pyrilamine, triprolidine

REFERENCE
Supelco Catalog, **1994**, 768.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotro-

pine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 11.35

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.80 (A), 5.25 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopen-

tal, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Sumchiral CSP 8 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:10:1
Flow rate: 1
Detector: UV 230-280

CHROMATOGRAM
Retention time: 33, 45 (enantiomers)

KEY WORDS
chiral

REFERENCE
Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, *694*, 129–134.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.09

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM
Retention time: k' 5.9
Internal standard: N-ethylnordiazepam (k' 2.1)
Limit of detection: 300 ng/mL

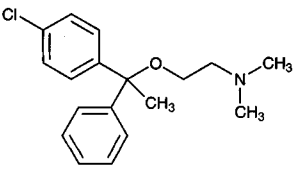
OTHER SUBSTANCES
Extracted: caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, hydromorphone, hydrocodone
Interfering: codeine

KEY WORDS
column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Chlorphenoxamine



Molecular formula: $C_{18}H_{22}ClNO$
Molecular weight: 303.83
CAS Registry No.: 77-38-3, 562-09-4 (HCl)
Merck Index: 2234
Lednicer No.: 1 44

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

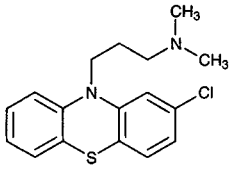
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Chlorpromazine



Molecular formula: $C_{17}H_{19}ClN_2S$
Molecular weight: 318.87
CAS Registry No.: 50-53-3, 69-09-0 (HCl)
Merck Index: 2238
Lednicer No.: 1 319

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL 50 μg/mL mesoridazine in water + 100 μL 1 M HCl, vortex for 30 s, add 4 mL isopropanol, mix for 5 min, centrifuge at 5000 rpm at 0° for 20 min. Remove the supernatant and adjust the pH to 12.5 with 200 μL 5 M NaOH, mix for 10 s, add 4 mL n-heptane, mix for 10 min, centrifuge at 2500 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, mix for 2 min, inject a 75-100 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.2 5 μm Spherisorb CN
Mobile phase: MeCN:15 mM pH 6.5 acetate buffer 90:10
Flow rate: 1.6
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM

Retention time: 1.9
Internal standard: mesoridazine (7)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Midha,K.K.; Cooper,J.K.; McGilveray,I.J.; Butterfield,A.G.; Hubbard,J.W. High-performance liquid chromatographic assay for nanogram determination of chlorpromazine and its comparison with a radioimmunoassay, *J.Pharm.Sci.*, **1981**, *70*, 1043–1046.

SAMPLE

Matrix: blood
Sample preparation: 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:5 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 21.7

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, thioridazine, thiothixene, trifluoperazine, trifluopromazine, trihexyphenidyl, trimeprazine, metabolites

KEY WORDS

plasma

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 1 mL 100 ng/mL prochlorperazine in water, vortex for 10 s, add 500 μL saturated sodium carbonate, vortex, add 5 mL pentane:isopropanol 97:3, mix for 20 min, centrifuge at 1725 g for 5 min, remove the organic layer and repeat the extraction. Combine the organic layers and evaporate them to dryness at 65° after adding a few anti-bumping granules. Cool, add 200 μL MeCN, mix for 20 s, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Spherisorb CN
Mobile phase: MeCN:100 mM ammonium acetate 90:10
Flow rate: 4
Injection volume: 100
Detector: E, Bioanalytical Systems Model LC4A, glassy carbon electrode +0.9 V, fixed 10 nA feed

CHROMATOGRAM

Retention time: 2.4
Internal standard: prochlorperazine (4.4)
Limit of detection: 0.1 ng/mL
Limit of quantitation: 0.25 ng/mL

KEY WORDS

plasma

REFERENCE

Cooper,J.K.; McKay,G.; Midha,K.K. Subnanogram quantitation of chlorpromazine in plasma by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1983**, *72*, 1259–1262.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane:isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)

Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.23
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES
Extracted: amitriptyline, chlordiazepoxide, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, haloperidol, imipramine, nortriptyline, oxazepam, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine

KEY WORDS
plasma

REFERENCE
Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 200 μL 5 M NaOH + 10 mL chloroform, shake for 10 min, stand in an ice bath for at least 30 min, centrifuge at 4° at 2800 rpm (RCF = 1578) for 10 min. Remove the organic layer and evaporate it under nitrogen at 40°. Dissolve the residue in 200 μL mobile phase, inject.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb nitrile
Mobile phase: MeCN:100 mM $(NH_4)H_2PO_4$ + 50 mg/L EDTA adjusted to pH 6.5 with ammonia 60:40
Flow rate: 2
Injection volume: 200
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode 0.85 V

CHROMATOGRAM
Retention time: 4
Internal standard: chlorpromazine

OTHER SUBSTANCES
Simultaneous: prochlorperazine

KEY WORDS
plasma; chlorpromazine is IS

REFERENCE
Fowler,A.; Taylor,W.; Bateman,D.N. Plasma prochlorperazine assay by high-performance liquid chromatography-electrochemistry, *J.Chromatogr.*, **1986**, *380*, 202–205.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL water, vortex for a few s, add 500 μL 650 mM sodium carbonate, vortex for a few s, add 7 mL pentane:ethyl acetate 75:25, mix for 15 min, let stand for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 65°, reconstitute the residue in 40 μL MeCN, mix for a few s, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Spherisorb cyano
Mobile phase: MeCN:MeOH:100 mM pH 7 ammonium acetate 90:5:5
Flow rate: 1.5
Injection volume: 30
Detector: E, ESA 5100A, Model 5020 guard cell +1.00 V, model 5011 analytical cell, cell 1 +0.50 V, cell 2 +0.75 V

CHROMATOGRAM
Retention time: 10.87
Internal standard: chlorpromazine

OTHER SUBSTANCES
Extracted: fluphenazine

KEY WORDS
plasma; chlorpromazine is IS

REFERENCE
Cooper,J.K.; Hawes,E.M.; Hubbard,J.W.; McKay,G.; Midha,K.K. An ultrasensitive method for the measurement of fluphenazine in plasma by high-performance liquid chromatography with coulometric detection, *Ther.Drug Monit.*, **1989**, *11*, 354–360.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 650 mM sodium carbonate, vortex, add 7 mL pentane:ethyl acetate 50:50, shake vigorously for 15 min, centrifuge at 1110 *g* for 10 min. Remove the organic layer and evaporate it to dryness at 65° under nitrogen. Reconstitute the residue in 300 μL MeCN:MeOH:isopropanol:water: 1 M ammonium acetate pH 5.0 83:5:5:6.65:0.35, sonicate for 5 min, vortex for 30 s, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Burdick & Jackson CN
Mobile phase: MeCN:MeOH:isopropanol:water: 1 M ammonium acetate pH 7.2 83:5:5:6.65: 0.35
Flow rate: 1.5
Injection volume: 100
Detector: E, ESA Model 5100A Coulochem detector, Model 5011 analytical cell, detector 1 +0.50 V, detector 2 +0.70 V

CHROMATOGRAM
Retention time: 10.2
Internal standard: chlorpromazine

OTHER SUBSTANCES
Simultaneous: promethazine

KEY WORDS
serum; chlorpromazine is IS

REFERENCE

Fox,A.R.; McLoughlin,D.A. Rapid, sensitive high-performance liquid chromatographic method for the quantification of promethazine in human serum with electrochemical detection, *J.Chromatogr.*, **1993**, *631*, 255–259.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spherisorb cyano

Column: 250 × 4.6 5 μm Ultrasphere cyano

Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)

Flow rate: 1

Injection volume: 20

Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM

Retention time: 26.4

Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES

Extracted: clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, imipramine, pipamperone, risperidone, trihexyphenidyl

Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS

plasma; SPE

REFERENCE

Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE

Matrix: blood

Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 7.0
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: norchlorimipramine

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8

Injection volume: 50
Detector: UV 256

CHROMATOGRAM

Retention time: 11.50
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH: water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES

Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 230, 254

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, pindolol, zolpidem
Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 1 mL Serum or milk + 5 μL 100 μg/mL methotrimeprazine in MeOH + 5 mL 0.5 (serum) or 1 (milk) M HCl, mix, add to the SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, elute with 5 mL MeOH:water 60:40, evaporate eluate to dryness under vacuum at 60°, dissolve residue in 100 μL mobile phase, inject whole amount.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Develosil C8-5 (Nomura)
Mobile phase: MeCN:0.5% KH_2PO_4 adjusted to pH 4.5 with 50% phosphoric acid 35:65
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 13
Internal standard: methotrimeprazine (levomepromazine) (10)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
serum; SPE

REFERENCE
Ohkubo,T.; Shimoyama,R.; Sugawara,K. Determination of chlorpromazine in human breast milk and serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 328–332.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 39.19
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexiphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 4 mL water + 1 mL 160 ng/mL thioridazine + 0.8 mL 1 M NaOH + 15 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge at 700 g, remove the organic layer, repeat the extraction twice more. Combine

the organic layers and evaporate them to dryness, reconstitute the residue in 10 mL 50 mM HCl, add 20 mL diethyl ether, shake for 3 min. Remove the aqueous layer and make it alkaline with 1 mL 5 M NaOH, add 10 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge at 700 g. Remove the organic layer and evaporate it to dryness, dissolve the residue in 1 mL MeCN, inject a 50 μL aliquot. Urine. 20 mL Urine + 3 mL 1 M HCl + 1 mL 1 μg/mL thioridazine, wash with diethyl ether, make the aqueous layer alkaline with 5 M NaOH, add n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge at 700 g. Remove the organic layer and evaporate it to dryness, dissolve the residue in 1 mL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:pyridine:THF:100 mM pH 3.5 acetate buffer 68.9:0.1:1:30 containing 20 mM sodium perchlorate
Flow rate: 0.7
Injection volume: 10-50
Detector: E, Yanaco Model VMD-101, glassy carbon working electrode 0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.0
Internal standard: thioridazine (9.5)
Limit of detection: 0.5 ng/mL (urine), 2 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: methotrimeprazine (levomepromazine)

KEY WORDS

plasma

REFERENCE

Murakami,K.; Ueno,T.; Hijikata,J.; Shirasawa,K.; Muto,T. Simultaneous determination of chlorpromazine and levomepromazine in human plasma and urine by high-performance liquid chromatography using electrochemical detection, *J.Chromatogr.*, **1982**, *227*, 103–112.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 254.7

CHROMATOGRAM
Retention time: 16.035

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablet or capsule, to 2 mg amitriptyline add 20 mL MeOH, shake 30 min, centrifuge at 2000 rpm for 5 min, to 5 mL supernatant add 4 mL 1.25 mg/mL norephedrine.HCl in MeOH, dilute to 10 mL with MeOH.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:MeOH:25 mM pH 4.8 sodium acetate-acetic acid buffer 35:45:20
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.1
Internal standard: Norephedrine (2.7)

OTHER SUBSTANCES
Also analyzed: amitriptyline, imipramine, thioridazine, trifluoperazine

KEY WORDS
tablets; capsules

REFERENCE
Lovering,E.G.; Beaulieu,N.; Lawrence,R.C.; Sears,R.W. Liquid chromatographic method for identity, assay, and content uniformity of five tricyclic drugs, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 168–171.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Zorbax ODS
Mobile phase: MeOH containing 0.5 g/L sodium acetate
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 5.0

OTHER SUBSTANCES
Simultaneous: trifluoperazine, trihexyphenidyl

REFERENCE

Pradas,T.N.V.; Sivakumar,M. HPLC quantification of a tricomponent psychiatric formulation containing chlorpromazine, trifluoperazine and trihexyphenidyl, *Pharmazie*, **1992**, *47*, 231–231.

SAMPLE

Matrix: hair

Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Nova-Pak phenyl
Mobile phase: MeCN:buffer 55:45 (Buffer was 10 mM pH 3.0 KH_2PO_4.)
Flow rate: 1.5
Injection volume: 30
Detector: UV 255

CHROMATOGRAM

Internal standard: protriptyline (UV 214) (4)

OTHER SUBSTANCES

Extracted: thioridazine (UV 265)

REFERENCE

Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm LiChrosphere 100RP-18
Mobile phase: MeOH:25% (w/w) ammonia 99.2:0.8
Flow rate: 1.2
Injection volume: 20
Detector: UV 258

CHROMATOGRAM

Retention time: k' 2.78

OTHER SUBSTANCES

Simultaneous: carbetapentane

REFERENCE

Gad-Kariem,E.A.; Abounassif,M.A. Determination of pentoxyverine in cough preparations by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3049–3059.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55

Flow rate: 0.6
Injection volume: 20
Detector: UV 255

OTHER SUBSTANCES

Also analyzed: amitriptyline, clomipramine, promazine, promethazine, thymol

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen,

procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: mesoridazine, promethazine, acetophenazine, thioridazine, prochlorperazine, butaperazine, thiethylperazine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 18.9

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: mesoridazine, promazine, thiothixene, trifluoperazine, thioridazine
Also analyzed: amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenor-

phine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 17.00 (A), 7.75 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50-200 μL aliquot of a solution in pH 7.4 Tyrode's buffer.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C-18
Mobile phase: MeCN:50 mM phosphoric acid:triethylamine 40:60:0.1
Column temperature: 35
Flow rate: 0.6
Injection volume: 50-200
Detector: UV 230

OTHER SUBSTANCES

Also analyzed: propantheline, verapamil

KEY WORDS
buffer

REFERENCE
Saitoh,H.; Aungst,B.J. Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine, *Pharm.Res.*, **1995**, *12*, 1304–1310.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 25 ng/mL solution in pH 4.0 acetate/citrate buffer.

HPLC VARIABLES
Column: 150 × 0.32 3 μm Hypersil C18
Mobile phase: MeCN:pH 4.0 acetate/citrate buffer 45:55
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: methotrimeprazine (levomepromazine), thioridazine

KEY WORDS
microcolumn

REFERENCE
Streel,B.; Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Injection-generated solvent and pH gradients for sample enrichment on injection of large volumes in microcolumn liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 254–256.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 10.23

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES
Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 25
Limit of detection: 6 ng/g

OTHER SUBSTANCES
Extracted: azaperol, carazolol, acepromazine, xylazine, azaperone, haloperidol, propiomazine

KEY WORDS
SPE; pig; kidney

REFERENCE
Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to

the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 µL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 µL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES

Guard column: Hypersil 5 µm SAS C1
Column: 250 mm long 5 µm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM

Retention time: 32
Limit of detection: 2 ng/g

OTHER SUBSTANCES

Extracted: azaperol, acepromazine, carazolol, azaperone, xylazine, haloperidol, propiomazine

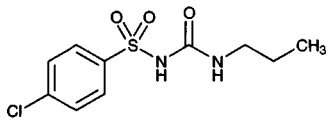
KEY WORDS

SPE; pig; kidney; liver

REFERENCE

Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

Chlorpropamide



Molecular formula: $C_{10}H_{13}ClN_2O_3S$
Molecular weight: 276.74
CAS Registry No.: 94-20-2
Merck Index: 2239
Lednicer No.: 1 137

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 ng tolbutamide + 500 µL 1 M HCl + 8 mL chloroform, shake on a reciprocal shaker, shake for 10 min in a reciprocal shaker, centrifuge at 2000 g for 15 min. Remove 7 mL of the lower organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 µL 3 mg/mL dinitrofluorobenzene in n-butyl acetate, heat at 120° for 30 min, evaporate to dryness under a stream of nitrogen at 60°, dissolve the residue in 100 µL mobile phase, inject a 30-70 µL aliquot. (Recrystallize dinitrofluorobenzene from diethyl ether. Prepare solutions weekly, store at 4° in the dark.)

HPLC VARIABLES

Column: 125 × 4.6 5 µm C8 (Perkin-Elmer)
Mobile phase: MeCN:water 50:50 containing 0.15% phosphoric acid
Flow rate: 1.5
Injection volume: 30-70
Detector: UV 350

CHROMATOGRAM

Retention time: 6.2
Internal standard: tolbutamide (4.5)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: glyburide
Noninterfering: acetaminophen, aspirin, diazepam, chlordiazepoxide, quinidine, phenytoin, theophylline, phenobarbital

KEY WORDS

plasma; derivatization

REFERENCE

Zecca,L.; Trivulzio,S.; Pinelli,A.; Colombo,R.; Tofanetti,O. Determination of glibenclamide, chlorpropamide and tolbutamide in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1985**, *339*, 203–209.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 2 mL water + 200 µL 1 (?) M HCl + 200 µL 2.5 µg/mL glibornuride in MeOH + 7 mL diethyl ether, mix, centrifuge at 2000 rpm for 5 min. Remove 6.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 µL 2 mg/mL dinitrofluorobenzene in butyl acetate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 µL mobile phase, inject a 120 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb ODS 2

Mobile phase: MeCN:0.4% aqueous phosphoric acid 75:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 120
Detector: UV 360

CHROMATOGRAM
Retention time: 4.2
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: glyburide (glibenclamide), glipizide, tolazamide, tolbutamide

KEY WORDS
serum; derivatization; serum

REFERENCE
Starkey,B.J.; Mould,G.P.; Teale,J.D. The determination of sulphonylurea drugs by HPLC and its clinical application, *J.Liq.Chromatogr.*, **1989**, *12*, 1889–1896.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 2 mL diethyl ether, vortex 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Decant ether layer and evaporate it to dryness under a stream of nitrogen at 35-40°. Reconstitute extract in 100 μL mobile phase, vortex 30 s, inject 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:10 mM orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 25-50
Detector: UV 230

CHROMATOGRAM
Retention time: 6.09
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: glyburide, gliclazide, glipizide, tolbutamide, tolazamide
Noninterfering: trimethoprim, sulfamethoxazole

KEY WORDS
plasma

REFERENCE
Shenfield,G.M.; Boutagy,J.S.; Webb,C. A screening test for detecting sulfonylureas in plasma, *Ther.Drug Monit.*, **1990**, *12*, 393–397.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 100 μg/mL tolbutamide in water + 500 μL 100 mM HCl + 3 mL dichloromethane, mix for 15 s, centrifuge. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 1000 × 8 10 μm radial pak C18 (Waters)
Mobile phase: MeOH:0.2% acetic acid 60:40 adjusted to pH 6.7 with 1 M NaOH (Wash with MeCN at 1 mL/min for 20 min at the end of each day.)
Flow rate: 0.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 8
Internal standard: tolbutamide (11)
Limit of detection: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Bakare,M.T.; Mustapha,A.; Abdu-Aguye,I. An improved high-performance liquid chromatographic determination of chlorpropamide in human plasma, *Chromatographia*, **1994**, *39*, 107–109.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 3.85
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazo-

cine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μL Plasma or 200 μL urine + 100 μL water + 100 μL 1 M HCl + 4 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 2-3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeCN:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 7 μm BST C8 (BST, Budapest)
Mobile phase: MeCN:isopropanol:0.1% orthophosphoric acid 17:17:66
Flow rate: 1.2
Injection volume: 5
Detector: UV 235

CHROMATOGRAM

Retention time: 8.5
Internal standard: chlorpropamide

OTHER SUBSTANCES

Extracted: tolbutamide

KEY WORDS

plasma; chlorpropamide is IS

REFERENCE

Csillag,K.; Vereczkey,L.; Gachalyi,B. Simple high-performance liquid chromatographic method for the determination of tolbutamide and its metabolites in human plasma and urine using photodiode-array detection, *J.Chromatogr.*, **1989**, *490*, 355–363.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 17.657

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Grind tablets, weigh out amount equivalent to 45-55 mg chlorpropamide, add 70-80 mL mobile phase, shake for 6-8 min, make up to 100 mL with mobile phase, dilute an aliquot to 0.05 mg/mL with mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5-6 μm Zorbax ODS

Mobile phase: MeCN:1% acetic acid 48:52

Flow rate: 1.5

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: k' 1.8

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

rugged; tablets

REFERENCE

Everett,R.L. Liquid chromatographic determination of chlorpropamide in tablet dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 519–521.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline,

tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.40 (A), 6.34 (B)

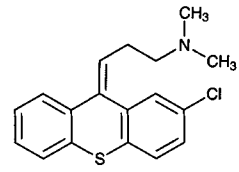
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Chlorprothixene



Molecular formula: $C_{18}H_{18}ClNS$
Molecular weight: 315.87
CAS Registry No.: 113-59-7
Merck Index: 2241
Lednicer No.: 1 399

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 5 µg/mL thioridazine + 2 mL water + 2 mL 2 M NaOH, mix well, add 10 mL heptane:isoamyl alcohol 99:1, shake slowly on a reciprocating shaker for 15 min, centrifuge at 5-10° at 1207 g for 5 min. Remove 8.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 µL MeCN:water 60:40, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm LC-PCN (cyano) (Supelco)
Mobile phase: MeCN:20 mM pH 4.5 KH_2PO_4 60:40
Column temperature: 40
Flow rate: 2
Injection volume: 50
Detector: UV 229 or E, IBM Model 230, Model 3892 glassy carbon electrode, 1000 mV vs saturated calomel electrode

CHROMATOGRAM

Retention time: 7.6
Internal standard: thioridazine (8.5)
Limit of quantitation: 5 ng/mL (UV)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; UV and electrochemical detection have about same sensitivity

REFERENCE

Brooks,M.A.; DiDonato,G.; Blumenthal,H.P. Determination of chlorprothixene and its sulfoxide metabolite in plasma by high-performance liquid chromatography with ultraviolet and amperometric detection, *J.Chromatogr.*, **1985**, *337*, 351–362.

SAMPLE

Matrix: blood
Sample preparation: Work under yellow light. 1 mL Serum + 2 mL water + 2 mL 2 M NaOH, vortex for 10 s, add 5 mL water-saturated n-heptane:isoamyl alcohol 99:1, shake gently for 20 min, centrifuge at 4° at 2800 g, remove organic layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under reduced pressure. Dissolve the residue in 500 µL MeCN, inject a 30 µL aliquot (store at 5°).

HPLC VARIABLES

Column: 250 × 4.6 5 µm Nucleosil 100 CN
Mobile phase: MeCN:pyridine:140 mM sodium acetate pH 3.1 698:2:300
Flow rate: 0.9
Injection volume: 100

Detector: E, Environmental Sciences Assoc. Coulochem II, Model 5011 detector cell, oxidative screen mode, screen electrode +0.5 V, sample electrode +0.85 V

CHROMATOGRAM
Retention time: 11
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: promethazine, methotrimeprazine (levomepromazine)

KEY WORDS
serum; recirculate mobile phase

REFERENCE
Bagli,M.; Rao,M.L.; Höflich,G. Quantification of chlorprothixene, levomepromazine and promethazine in human serum using high-performance liquid chromatography with coulometric electrochemical detection, *J.Chromatogr.B*, **1994**, *657*, 141–148.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Whole blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry under suction, wash with 2 mL acetone:chloroform 50:50, elute with 3 mL freshly prepared ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm Asahipak ODP-50
Mobile phase: MeCN:50 mM ammonium acetate 85:15
Flow rate: 0.6
Injection volume: 10
Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, Finnigan MAT TSP-2 interface, collision gas argon 3.5 mTorr, collision offset -17.5 V, repeller 70 V, vaporizer 130-5°, source 200°, filament off, multiplier 1500 V, dynode power 15 kV, scantime 1.20 s, MSMSC factor 0, monitor 316–271. (The effluent from the column was mixed with 50 mM ammonium acetate pumped at 0.6 mL/min. The mixture flowed to the detector.)

CHROMATOGRAM
Retention time: 4.50
Limit of detection: 0.1 ng

OTHER SUBSTANCES
Extracted: flupenthixol, thiothixene, zuclopenthixol

KEY WORDS
whole blood; SPE

REFERENCE
Verweij,A.M.A.; Hordijk,M.L.; Lipman,P.J.L. Quantitative liquid chromatography, thermospray/tandem mass spectrometric (LC/TSP/MS/MS) analysis of some tranquilizers of the thioxanthene group in whole-blood, *J.Liq.Chromatogr.*, **1994**, *17*, 4009–4110.

SAMPLE
Matrix: bulk
Sample preparation: Prepare solutions in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4 3 μm Hypersil C18-BDS
Mobile phase: MeCN:MeOH:water 40:5:55 containing 6 g/L KH_2PO_4, 2.9 g/L sodium lauryl sulfate, and 9 g/L tetra-*n*-butylammonium bromide
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Retention time: 8.66
Limit of detection: 300 ng/mL
Limit of quantitation: 900 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Duignan,G.M.; Miller,J.H.M.B.; Skellern,G.G. Development of a liquid chromatographic method for the control of related substances in chlorprothixene hydrochloride, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 451–456.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine,

metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 202, 225, 264, 324

CHROMATOGRAM

Retention time: 6.0
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, carbamazepine, chlordiazepoxide, clonazepam, caffeine, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50

Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: triflupromazine, carphenazine, methotrimeprazine, promazine, perphenazine, deserpidine, thiothixene, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 17.60 (A), 8.29 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, pra-

zosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

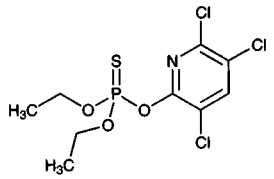
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Chlorpyrifos



Molecular formula: $C_9H_{11}Cl_3NO_3PS$
Molecular weight: 350.59
CAS Registry No.: 2921-88-2
Merck Index: 2242

SAMPLE

Matrix: bile, blood, tissue, urine
Sample preparation: Blood. Extract three time with MeCN:phosphoric acid 99:1, centrifuge. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute, inject an aliquot. Urine. Inject directly. Bile. Dilute 1:4 with water, inject an aliquot. Tissue. Homogenize whole fish, extract three times with 10 mL acetone:phosphoric acid 99:1. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute, inject an aliquot.

HPLC VARIABLES

Column: RCM 100 C18 (Waters)
Mobile phase: Gradient. A was MeCN:water:phosphoric acid 10:89:1. B was MeCN:phosphoric acid 99:1. A:B 0:100 for 3 min, to 25:75 over 12 min, maintain at 25:75 for 10 min.
Flow rate: 1.5
Injection volume: 200
Detector: UV 280

CHROMATOGRAM

Retention time: 23

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

fish; catfish; pharmacokinetics

REFERENCE

Barron,M.G.; Plakas,S.M.; Wilga,P.C. Chlorpyrifos pharmacokinetics and metabolism following intravascular and dietary administration in channel catfish, *Toxicol.Appl.Pharmacol.*, **1991**, *108*, 474–482.

SAMPLE

Matrix: blood
Sample preparation: 1.5 mL Serum + 2 mL 200 mM pH 7.0 phosphate buffer, add to an Extrelut No. 3 SPE column, let stand for 10 min, elute with 15 mL n-hexane:diethyl ether 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 150 µL MeOH:water 70:30, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: Gradient. MeOH:water from 70:30 to 90:10.
Flow rate: 1
Injection volume: 200
Detector: MS, Hitachi Model M-2000, APCI non-equilibrium interface, vaporizer 250°, nebulizer 400°, ionization needle electrode current 5 µA, drift voltage 230 V, vacuum 0.0001 Pa, ion-source slit 500 µm, collector slit 400 µm, accelerated electrical potential 4 kV, secondary electronic step-up tube potential 1.3 kV, negative-ion mode

CHROMATOGRAM
Retention time: 17
Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: chlorpyrifos-methyl, disulfoton, EPN, ethion, fenitrothion, methidathion, parathion, parathion-methyl

KEY WORDS
serum; SPE; m/z 330

REFERENCE
Kawasaki,S.; Ueda,H.; Itoh,H.; Tadano,J. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1992**, *595*, 193–202.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize mouse brain in ten volumes 100 mM pH 7.4 sodium phosphate buffer. 2 mL Plasma or homogenate + 1 g NaCl + 2 mL ethyl acetate + coumaphos, vortex for 30 s, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen.

HPLC VARIABLES
Column: 300 × 4 μPorasil
Mobile phase: Dichloromethane:glacial acetic acid 100:0.02
Flow rate: 1
Detector: UV 290

CHROMATOGRAM
Retention time: 3.3
Internal standard: coumaphos (10.0)
Limit of detection: 40 ng

OTHER SUBSTANCES
Extracted: parathion

KEY WORDS
plasma; rat; mouse; microsomes; brain; normal phase

REFERENCE
Sultatos,L.G.; Costa,L.G.; Murphy,S.D. Determination of organophosphorus insecticides, their oxygen analogs and metabolites by high pressure liquid chromatography, *Chromatographia*, **1982**, *15*, 669–671.

SAMPLE
Matrix: food
Sample preparation: 30 g Rice + 50 mL MeOH, let stand for 48 h with occasional manual shaking, remove a 1 mL aliquot of the MeOH layer and evaporate it to near dryness under a stream of nitrogen, add 1 mL hexane, shake, repeat extraction. Combine the hexane layers and add them to a Sep-Pak Florisil SPE cartridge, elute with 3 mL acetone:hexane 40:60. Combine the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak (Waters)
Column: 250 × 3.9 Nova-Pak C18

Mobile phase: Gradient. MeCN:water from 40:60 to 70:30 over 12 min or Isocratic MeCN: water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 18 (gradient), 10 (isocratic) (for chlorpyrifos-methyl)
Limit of detection: 600 ng/g

OTHER SUBSTANCES
Extracted: methacrifos, fenitrothion, etrimfos, carbaryl, pirimphos-methyl (UV 247)

KEY WORDS
rice; SPE

REFERENCE
Brayan,J.G.; Haddad,P.R.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *447*, 249–255.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 313

CHROMATOGRAM
Retention time: 0.61

OTHER SUBSTANCES
Also analyzed: amitraz (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), permethrin (UV 229), phosmet (UV 229)

REFERENCE
Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

SAMPLE
Matrix: soil
Sample preparation: 20 g Air-dried soil + 2 mL water, mix, add 50 mL acetone, shake mechanically at 280 excursions/min for 30 min, filter (0.45 μm), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 5 μm Spherex amino-derivatized silica (Phenomenex)
Mobile phase: Hexane:THF 97:3
Flow rate: 1.5
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Limit of quantitation: 50 ppb

KEY WORDS
comparison with GC/MS and immunoassay

REFERENCE
Hill,A.S.; Skerritt,J.H.; Bushway,R.J.; Pask,W.; Larkin,K.A.; Thomas,M.; Korth,W.; Bowmer,K. Development and application of laboratory and field immunoassays for chlorpyrifos in water and soil matrices, *J.Agric.Food Chem.*, **1994**, *42*, 2051–2058.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize mouse brain with 10 volumes 100 mM pH 7.4 sodium phosphate buffer. 2 mL Homogenate + 1 g NaCl + 2 mL coumaphos in ethyl acetate, vortex for 30 s, centrifuge at 1000 g for 10 min, repeat extraction with 2 mL ethyl acetate. Combine the ethyl acetate layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: μPorasil
Mobile phase: Dichloromethane:glacial acetic acid 100:0.02
Flow rate: 1
Detector: UV 290

CHROMATOGRAM
Internal standard: coumaphos

KEY WORDS
mouse; brain; normal phase; pharmacokinetics

REFERENCE
Sultatos,L.G.; Costa,L.G.; Murphy,S.D. Factors involved in the differential acute toxicity of the insecticides chlorpyrifos and methyl chlorpyrifos in mice, *Toxicol.Appl.Pharmacol.*, **1982**, *65*, 144–152.

SAMPLE
Matrix: water
Sample preparation: Condition a C8 SPE cartridge with 10 mL MeOH and 10 mL water. Acidify water to pH 2.2 with concentrated HCl, filter (0.45 μm). Add 100 mL water to the SPE cartridge at 5-6 mL/min, dry under vacuum for 30 min, elute with four 500 μL portions of MeOH at 5-6 mL/min, make up the volume of the eluate to 2 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 20 mm long 37-75 μm Porasil B
Column: 250 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeOH:water 82:18
Flow rate: 1
Injection volume: 20-25
Detector: UV 230

CHROMATOGRAM
Retention time: 14.6
Limit of detection: 5 ppb

OTHER SUBSTANCES
Extracted: pendimethalin

KEY WORDS
SPE

REFERENCE
Bogus,E.R.; Watschke,T.L.; Mumma,R.O. Utilization of solid-phase extraction and reversed-phase and ion-pair chromatography in the analysis of seven agrochemicals in water, *J.Agric.Food Chem.*, **1990**, *38*, 142–144.

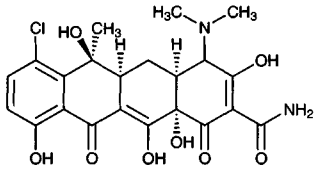
SAMPLE
Matrix: water
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 5 mL MeOH and 10 mL water. Add 250 mL water to the SPE cartridge, elute with 2 mL ethyl acetate, inject a 25 μL aliquot.

HPLC VARIABLES
Column: Ultremex C18
Mobile phase: MeCN:water 80:20
Flow rate: 1.1
Injection volume: 25
Detector: UV 224

KEY WORDS
SPE

REFERENCE
Hill,A.S.; Skerritt,J.H.; Bushway,R.J.; Pask,W.; Larkin,K.A.; Thomas,M.; Korth,W.; Bowmer,K. Development and application of laboratory and field immunoassays for chlorpyrifos in water and soil matrices, *J.Agric.Food Chem.*, **1994**, *42*, 2051–2058.

Chlortetracycline



Molecular formula: $C_{22}H_{23}ClN_2O_8$
Molecular weight: 478.89
CAS Registry No.: 57-62-5, 64-72-2 (HCl)
Merck Index: 2245
Lednicer No.: 1 212

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 200 μL 24% trichloroacetic acid in MeOH + 300 μL mobile phase buffer (A), vortex for 1 min, centrifuge at 2000 g for 15 min, inject 50 μL of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Capcell C18 type SG-120 (Shiseido)
Mobile phase: MeOH:buffer 55:45 (Buffer (A) was 100 mM pH 6.5 sodium acetate containing 35 mM calcium chloride and 25 mM disodium ethylenediamine tetraacetate.)
Column temperature: 30 ± 0.2
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 512

CHROMATOGRAM

Retention time: 11
Limit of detection: 35 ng/mL

OTHER SUBSTANCES

Also analyzed: tetracycline, oxytetracycline

KEY WORDS

serum

REFERENCE

Iwaki,K.; Okumura,N.; Yamazaki,M. Rapid determination of tetracycline antibiotics in serum by reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1993**, *619*, 319–323.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 1 mL buffer, mix for 30 s, add 6 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. Urine. 100 μL Urine + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 400 μL buffer, mix for 30 s, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. (Buffer was 27.6 g NaH_2PO_4 + 25.2 g sodium sulfite in 100 mL water, pH 6.1.)

HPLC VARIABLES

Column: 100 × 2 5 μm Lichrosorb RP8

Mobile phase: MeCN:100 mM citric acid 24:76
Flow rate: 0.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 7
Internal standard: demeclocycline (4)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: oxytetracycline, tetracycline, methacycline, doxycycline

KEY WORDS
serum

REFERENCE
De Leenheer,A.P.; Nelis,H.J.C.F. Doxycycline determination in human serum and urine by high-performance liquid chromatography, *J.Pharm.Sci.*, **1979**, *68*, 999–1002.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10 mg/mL solution of tetracycline hydrochloride in water, inject a 20 μL aliquot. Prepare a 10 mg/mL solution of tetracycline in 100 mM HCl, inject a 20 μL aliquot. Formulations. Shake 500 mg capsule blend with 15 mL water and 1 mL concentrated ammonia until solid has dissolved, make up to 50 mL with pH 4.0 phosphate buffer, let stand for 10 min, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: Gradient. A was MeCN:water:phosphoric acid 240:1650:27, adjust pH to 2.2 with 45% KOH, make up to 2000 with water. B was MeCN:water:phosphoric acid 440:1500:27, adjust pH to 2.2 with 25% KOH, make up to 2000 with water. A:B 100:0 for 10 min then 0:100 for 5 min.
Flow rate: 2.6
Injection volume: 20
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Simultaneous: impurities, tetracycline

REFERENCE
Muhammad,N.; Bodnar,J.A. Separation and quantitation of chlortetracycline, 4-epitetracycline, 4-epianhydrotetracycline, and anhydrotetracycline in tetracycline by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 928–930.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μg/mL solution in buffer, inject an aliquot. Capsules, tablets. Prepare a 1 mg/mL solution of capsule contents or crushed tablets in buffer, sonicate for 10 min, filter (0.45 μm), dilute with buffer, inject an aliquot. Ointment. Dissolve 250 mg ointment in 30 mL diethyl ether, extract with three 25 mL portions of 10 mM HCl, combine the extracts and make up to 100 mL with 10 mM HCl, inject an aliquot. (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Laboratories)
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

capsules; tablets; ointment

REFERENCE

Bryan,P.D.; Stewart,J.T. Chromatographic analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns with acidic mobile phases, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 675–692.

SAMPLE

Matrix: eggs, tissue
Sample preparation: Prepare a metal chelate affinity chromatography (MCAC) column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH: water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Condition an SBD-RPS extraction membrane (3M Company, St. Paul, MN) with 2 mL MeOH and 2 mL 100 mM HCl. Add 20 mL 100 mM pH 4.0 sodium succinate buffer to 3 g pig kidney, pig muscle, cow liver, or whole chicken egg, vortex for 1 min and shake for 10 min on a horizontal shaker. Add 20 mL MeOH, sonicate for 5 min and centrifuge at 2666 g for 10 min at 4°. Filter the supernatant through a Whatman 541 filter paper. Add the clear supernatant to the MCAC column. Wash sequentially with 2 mL 100 mM sodium succinate buffer, 2 mL water, 2 mL MeOH, 2 mL water, and with 500 μL McIlvaine-EDTA-NaCl buffer. Elute with 3 mL McIlvaine-EDTA-NaCl buffer and adjust the eluate to pH 1.3 with 400 μL 4 M HCl. Add the eluate directly to the extraction membrane to prevent crystallization of EDTA. Wash the membrane with 1 mL 100 mM HCl and elute with four 250 μL portions of MeOH:25% ammonia 97:3, evaporate the eluate to dryness under the nitrogen at 40°. Reconstitute the dry residue with 250 μL 10 mM oxalic acid in water, vortex, sonicate. Inject a 100 μL aliquot. (The sodium succinate buffer was 100 mM succinic acid, pH adjusted to 4.0 with 10 M NaOH. Prepare the McIlvaine buffer by dissolving 12.9 g citric acid monohydrate and 10.9 g Na_2HPO_4 in 1 L water. The McIlvaine-EDTA-NaCl buffer was 100 mM EDTA and 500 mM NaCl in McIlvaine buffer. Protect all solutions from light.)

HPLC VARIABLES

Guard column: 5 × 3.0 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 8 μ PLRP-S (Polymer Laboratories)
Mobile phase: Gradient. A was 10 mM oxalic acid in water adjusted to pH 2.0 with 4 M HCl. B was MeCN. A:B from 85:15 to 60:40 over 16 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water stored at 4°.)

CHROMATOGRAM

Retention time: 19

Limit of detection: 0.92 ng/g (pig kidney), 0.66 ng/g (pig muscle), 1.05 ng/g (cow liver), 0.39 ng/g (chicken egg)
Limit of quantitation: 4 ng/g (pig kidney)

OTHER SUBSTANCES
Extracted: doxycycline, oxytetracycline, tetracycline
Also analyzed: demeclocycline

KEY WORDS
cow; liver; pig; kidney; muscle; chicken; metal chelate affinity chromatography; MCAC; SPE; post-column reaction

REFERENCE
Croubels,S.M.; Vanoosthuyze,K.E.I.; Van Peteghem,C.H. Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg, *J.Chromatogr.B*, **1997**, *690*, 173–179.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Condition an Anagel-TSK Chelate-SPW column with 25 μL 50 mg/mL copper sulfate in water and 500 μL. Homogenize 2 g sliced chicken liver with 1.2 mL 1 M pH 4 citrate buffer and 12 mL ethyl acetate for 1 min. Homogenize 2 g sliced tissue with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 1 min. Shake 2 g blended egg with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 15 min. Centrifuge the mixture at 11000 rpm for 10 min, decant the supernatant, reextract the residue with two 12 mL portions of ethyl acetate. Add 10 g anhydrous sodium sulfate to the combined supernatants, swirl, let stand for 5-10 min, filter (Whatman 1PS phase-separating filter paper). Evaporate the filtrate to dryness or to an oily residue on a rotary evaporator under reduced pressure at 40°, reconstitute the residue in 2 mL MeOH by vortexing, filter (0.2 μm syringe filter). Add 1.5 mL of the filtrate to the Anagel column at 0.36 mL/min, wash with 500 μL water, 500 μL MeOH, and 500 μL water. Elute the contents of the Anagel column onto the analytical column with mobile phase A, after 11 min remove the Anagel column from the circuit, elute column B using gradient elution of mobile phase A:B, monitor the effluent from column B. (Prepare 1 M pH 4 or 5 citrate buffer as follows: dissolve 192 g citric acid in approximately 800 mL water, adjust pH value with 1 M NaOH and make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S
Column: 150 × 4.6 5 μm Polymer Labs PLRP-S
Mobile phase: Gradient. A:B 100:0 for 11 min, to 0:100 in 10 min, maintain at 0:100 for 10 min. A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid, and 10 mM EDTA).
Flow rate: 1
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 27.3
Limit of detection: 6 ng/g

OTHER SUBSTANCES
Extracted: tetracycline, oxytetracycline, demeclocycline

KEY WORDS
chicken; egg; metal chelate affinity chromatography; muscle; liver; salmon; trout; venison; SPE

REFERENCE

Cooper,A.D.; Stubbings,G.W.F.; Kelly,M.; Tarbin,J.A.; Farrington,W.H.H.; Shearer,G. Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products, *J.Chromatogr.A*, **1998**, *812*, 321–326.

SAMPLE

Matrix: feed

Sample preparation: Weigh out 10 or 20 g feed, add 100 mL acid-acetone solution, shake for 2 min. Adjust the pH to less than 1.2 with HCl, shake, repeat until the pH is stable, shake for 45 min. Centrifuge at 2500 rpm for 5 min and filter. Dilute the filtrate with acid-acetone solution, inject a 20 μL aliquot. (Acid-acetone solution was acetone:water:4 M HCl 65:30:5.)

HPLC VARIABLES

Guard column: C18 Alltima (Alltech)

Column: 150 × 4.6 5 μm C18 Alltima (Alltech)

Mobile phase: Gradient. A was MeOH. B was buffer. A:B 35:65 for 5 min, to 60:40 over 8 min, maintain at 60:40 for 7 min, re-equilibrate at initial conditions for 5 min (Buffer was 100 mM sodium acetate containing 50 mM calcium chloride and 25 mM disodium EDTA, adjusted to pH 6.5 with concentrated HCl or 40% NaOH.)

Flow rate: 1.5

Injection volume: 20

Detector: F ex 390 em 512

CHROMATOGRAM

Retention time: 12.5

Limit of quantitation: 2.5 μg/g

OTHER SUBSTANCES

Noninterfering: amprolium, arsanilic acid, decoquinate, monensin, oxytetracycline, roxarsone, sulfamethazine, sulfathiazole, penicillin

KEY WORDS

comparison with UV 365

REFERENCE

Houglum,J.E.; Larson,R.D.; Knutson,A. Assay of chlortetracycline in animal feeds by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 961–965.

SAMPLE

Matrix: feed

Sample preparation: 20 g Feed + 100 mL 4 M HCl:acetone:water 1:8:6, shake mechanically for 45 min, allow to settle for 10 min, centrifuge an aliquot at 3500 rpm for 10 min, filter the supernatant (A) (Millex-HV) (reject first 8-10 drops), add 3 mL filtrate to an unconditioned J.T. Baker C18 SPE cartridge, slowly force through, reject the first few drops, inject a 20 μL aliquot of the eluate. (If necessary wash 10 mL supernatant (A) + 3.6 mL water saturated with dichloromethane with 3.6 mL dichloromethane saturated with water, filter (Millex-HV) the aqueous layer, inject an aliquot.)

HPLC VARIABLES

Column: 300 × 3.9 Nova-Pak C18

Mobile phase: MeCN:MeOH:10 mM oxalic acid 1.5:1:3.5, adjust pH to 2.0 with 7.2 M HCl

Flow rate: 1

Injection volume: 20

Detector: UV 370

CHROMATOGRAM

Retention time: 5

KEY WORDS
SPE

REFERENCE
Holland,D.C.; Faul,K.C.; Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of chlortetracycline hydrochloride in ruminant and poultry/swine feeds, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 780–784.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve ointment in petroleum ether, add an equal volume of EtOH:water 70:30, dilute with MeOH to 100 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 5:95 containing 1.3 mM disodium citrate, 1 mM tetrabutylammonium bromide, 1.1 mM citric acid, and 8 mM EDTA.
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.43

OTHER SUBSTANCES
Simultaneous: anhydrotetracycline, demeclocycline, doxycycline, epianhydrotetracycline, oxytetracycline, quatrimycin, rolitetracycline, tetracycline

KEY WORDS
ointment

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: honey
Sample preparation: Condition a 500 mg Baker-10 C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 10 mL saturated aqueous disodium EDTA. Condition a 500 mg Baker-10 COOH cartridge with MeOH:ethyl acetate 10:90. Dissolve 25 g honey in 50 mL 100 mM pH 4.0 disodium EDTA-McIlvaine buffer, filter. Add the filtrate to the C18 SPE cartridge, wash with 20 mL water, wash with 400 μL ethyl acetate, air dry under vacuum for 5 min, elute with 50 mL MeOH:ethyl acetate 10:90. Add a 5 mL aliquot to the COOH SPE cartridge, wash with 5 mL MeOH (?), elute with 10 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Chemcosorb 3C8 (Chemco)
Mobile phase: MeCN:MeOH:10 mM aqueous oxalic acid 3:2:16, pH 3.0
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: demeclocycline (demethylchlortetracycline), doxycycline, methacycline, minocycline, oxytetracycline, tetracycline

KEY WORDS
SPE

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Suzuki,M. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey, *J.Chromatogr.*, **1987**, *400*, 253–261.

SAMPLE
Matrix: honey
Sample preparation: Prepare a 100 mg Baker 10 C18 cartridge by washing with MeOH, water, and 10 mL saturated aqueous Na_2EDTA. Dissolve 5 g honey in 20 mL 100 mM pH 4.0 Na_2EDTA-McIlvaine buffer, filter, apply to cartridge, wash with 20 mL water, air dry under vacuum for 5 min. Condition a Baker 10 COOH cartridge with ethyl acetate. Elute contents of C18 cartridge onto COOH cartridge with 50 mL ethyl acetate. Wash COOH cartridge with 10 mL MeOH, elute with 10 mL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Bakerbond C8
Mobile phase: MeOH:MeCN:10 mM aqueous oxalic acid 1:1.5:3
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Simultaneous: tetracycline, doxycycline, oxytetracycline

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Uchiyama,M.; Asukabe,H.; Mori,Y.; Suzuki,M. Improvement of chemical analysis of antibiotics. IX. A simple method for residual tetracyclines analysis in honey using a tandem cartridge clean-up system, *J.Chromatogr.*, **1987**, *389*, 417–426.

SAMPLE
Matrix: milk
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM
Retention time: 7.9

OTHER SUBSTANCES
Extracted: demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline

KEY WORDS
metal chelate affinity chromatography; cow; SPE

REFERENCE
Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE
Matrix: milk
Sample preparation: 2 mL Milk + 4 mL buffer, filter (Amicon CF-25 ultrafiltration membrane) while centrifuging at 20° at 1000 g for 1 h, suspend solids in 2 mL buffer and repeat filtration for 40 min. Combine filtrates and inject a 500 μL aliquot as soon as possible. (Buffer (McIlvaine) was prepared by mixing 625 mL 28.41 g/L Na_2HPO_4 and 1 L 21.01 g/L citric acid monohydrate. The buffer was also 100 mM in disodium EDTA and the final pH was 4.0 ± 0.1.)

HPLC VARIABLES
Column: 150 × 3.9 Novapak C18
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 10 min, re-equilibrate at 0:0:100 at 1.5 mL/min for 5 min and at 1 mL/min for 1 min. (Flush daily with 10 column volumes of water. Store column in MeOH:water 60:40, flush with water before use.)
Column temperature: 30
Flow rate: 1
Injection volume: 500
Detector: UV 360

CHROMATOGRAM
Retention time: 13.1
Limit of detection: 19.8 ng/mL
Limit of quantitation: 51.9 ng/mL

OTHER SUBSTANCES
Extracted: oxytetracycline, tetracycline

KEY WORDS
cow; protect from light; ultrafiltrate

REFERENCE
Thomas,M.H. Simultaneous determination of oxytetracycline, tetracycline, and chlortetracycline in milk by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 564–567.

SAMPLE

Matrix: milk

Sample preparation: Place 22 g 40 μm, 18% load, end-capped bulk C18 material (Analytichem) in a 50 mL syringe barrel, wash with 2 column volumes hexane, dichloromethane, and MeOH, vacuum aspirate until dry. 2 g Bulk C18 material + 50 mg disodium EDTA + 50 mg oxalic acid + 500 μL milk + 10 μL MeOH, blend gently in a glass mortar and pestle for 30 s, place the mixture in a 10 mL plastic syringe barrel plugged with a piece of filter paper. Compress column volume to 4.5 mL, add a 100 μL pipette tip on the column outlet to restrict the flow. Wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL MeCN:ethyl acetate 75:25. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 4 10 μm Micro Pak ODS
Mobile phase: MeCN:10 mM oxalic acid in water 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: 5.8
Limit of detection: 2 ng

OTHER SUBSTANCES

Extracted: tetracycline, oxytetracycline

KEY WORDS

cow; matrix solid-phase dispersion

REFERENCE

Long,A.R.; Hsieh,L.C.; Malbrough,M.S.; Short,C.R.; Barker,S.A. Matrix solid-phase dispersion (MSPD) isolation and liquid chromatographic determination of oxytetracycline, tetracycline, and chlortetracycline in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 379–384.

SAMPLE

Matrix: milk

Sample preparation: Prepare a column as follows. Swirl Chelating Sepharose Fast Flow resin (Pharmacia) in its bottle, add it to a polypropylene column to give a bed volume of 1.0-1.2 mL, wash 3 times with 2 mL portions of water, wash with 2 mL 10 mM copper sulfate, wash with two 2 mL portions of water. Centrifuge 5 mL milk at 10° at 1500 g for 15 min, remove the lower layer and add it to 10 mL succinate buffer, mix, centrifuge at 1500 g for 30 min, add the supernatant to the column. Wash with 2 mL succinate buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, wash with 700 μL citrate/phosphate buffer (be careful not to disturb bed), elute with 2.5 mL citrate/phosphate buffer (column is white and eluate is blue). Filter (Amicon Centricon 30, MW 30000 cut-off; pre-washed by centrifuging with 2 mL water) while centrifuging at 5000 g for 30-90 min, inject a 600 μL aliquot of the ultrafiltrate. (Prepare succinate buffer by dissolving 11.8 g succinic acid in 980 mL water, adjust pH to 4.0 with 10 M NaOH, make up to 1 L. Prepare the citrate/phosphate buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 11 min, return to initial conditions.

Flow rate: 1
Injection volume: 600
Detector: UV 355

CHROMATOGRAM
Retention time: 15.3
Limit of detection: 1.27 ng/mL
Limit of quantitation: 2.35 ng/mL

OTHER SUBSTANCES
Extracted: demeclocycline, doxycycline, methacycline, minocycline, oxytetracycline, tetracycline
Noninterfering: chloramphenicol, gentian violet, hydromycin B, ivermectin, spectinomycin, sulfa drugs

KEY WORDS
cow; SPE; ultrafiltrate

REFERENCE
Carson,M.C. Simultaneous determination of multiple tetracycline residues in milk using metal chelate affinity chromatography, *J.AOAC Int.*, **1993**, *76*, 329–334.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 1 mL 1 M HCl, mix, add 24 mL MeCN slowly with swirling over 30 s, let stand for 5 min, decant the clear supernatant through a plug of glass wool. 15 mL Filtrate + 15 mL dichloromethane + 30 mL hexane, mix, collect the aqueous layer. Extract the organic layer with 1 mL water. Combine the aqueous layers, make up to 4 mL with water, filter (13 mm, 0.45 μm, PVDF), inject a 1000 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)
Column: 150 × 4.6 5 μm PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)
Mobile phase: Gradient. MeCN:buffer 20:80 for 3 min, to 38:62 over 22 min, maintain at 38:62 for 5 min, return to initial conditions for 1 min, re-equilibrate for 9 min. (Buffer was 3.94 g potassium oxalate + 3.61 g oxalic acid + 1.22 g sodium decanesulfonate in 1 L water, pH 2.30.)
Flow rate: 1
Injection volume: 1000
Detector: UV 365

CHROMATOGRAM
Retention time: 23
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: tetracycline, oxytetracycline

REFERENCE
White,C.R.; Moats,W.A.; Kotula,K.L. Optimization of a liquid chromatographic method for determination of oxytetracycline, tetracycline, and chlortetracycline in milk, *J.AOAC Int.*, **1993**, *76*, 549–554.

SAMPLE
Matrix: milk
Sample preparation: Prepare a column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH:water 20:80 (Pharmacia) to a 150 × 10 glass

column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Centrifuge 10 mL milk at 2100 g for 5 min, decant the skimmed milk, rinse the tube with two 1 mL portions of water. Add 10 mL pH 4.0 buffer to the milk and rinses, sonicate for 3 min, filter (Whatman 541 paper) the supernatant. Add the filtrate to the column, wash with 2 mL pH 4.0 buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, add 700 μL EDTA buffer to the column, elute with 3 mL EDTA buffer, add 20 μL 25 μg/mL demeclocycline hydrochloride in MeOH to the eluate, inject a 100 μL aliquot. (Prepare pH 4.0 buffer by adjusting 100 mM succinic acid to pH 4.0 with 10 M NaOH. Prepare EDTA buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 29.2 g NaCl, and 100 mmoles EDTA in 1 L water.)

HPLC VARIABLES

Guard column: 5 × 3 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 5 μm 100 Å PLRP-S (Polymer Laboratories)
Mobile phase: MeCN:MeOH:buffer 15:10:60 (Buffer was 10 mM oxalic acid adjusted to pH 2.0 with 4 M HCl.)
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water.)

CHROMATOGRAM

Retention time: 11.7
Internal standard: demeclocycline (8.3)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: oxytetracycline, tetracycline

KEY WORDS

protect from light; cow; post-column reaction; derivatization; SPE; complexation

REFERENCE

Croubels,S.; Van Peteghem,C.; Baeyens,W. Sensitive spectrofluorimetric determination of tetracycline residues in bovine milk, *Analyst*, **1994**, *119*, 2713–2716.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 100
Detector: UV 370

CHROMATOGRAM

Retention time: 6.3

OTHER SUBSTANCES

Simultaneous: furazolidone, oxytetracycline, tetracycline

REFERENCE

Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 10 mM HCl, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 5 μm PLRP-S styrene-divinyl benzene copolymer (Polymer Laboratories)
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 355

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: oxytetracycline, tetracycline

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 μg/mL solution in 10 mM HCl.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 5 μm PLRP-S styrene-divinylbenzene copolymer (Polymer Laboratories)
Mobile phase: Gradient. MeCN:50 mM pH 2.0 oxalate buffer 15:85, for 3 min to 60:40 over 17 min, maintain at 60:40 for 5 min, return to initial conditions over 1 min, re-equilibrate for 9 min. (After use flush with water for 10 min, store in MeCN:water 60:40.)
Flow rate: 1
Detector: UV 355

CHROMATOGRAM
Retention time: 15.5

OTHER SUBSTANCES
Simultaneous: oxytetracycline, tetracycline

REFERENCE
White,C.R.; Moats,W.A.; Kotula,K.L. Comparative study of high performance liquid chromatographic methods for the determination of tetracycline antibiotics, *J.Liq.Chromatogr.*, **1993**, *16*, 2873–2890.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30

Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 6 mL 500 mg Bond-Elut C8 SPE cartridge with 6 mL MeOH, 6 mL water, and 2 mL buffer A. Condition a 6 mL SPE cartridge containing 3 g

wet XAD-2 resin with 10 mL MeOH, 10 mL water, and 2 mL buffer B. Homogenize (Ultra-Turrax) 2 g tissue with 20 mL succinate buffer for 1 min, centrifuge at 30 897 g for 15 min, filter (Whatman No. 1 paper) the supernatant, dilute 12 mL filtrate with 6 mL buffer B. For sheep liver add the diluted filtrate to the C8 SPE cartridge, wash with 10 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. For cow kidney add the diluted filtrate to the XAD-2 cartridge, wash with 14 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. Inject 25 μL 50 mg/mL copper sulfate and 500 μL water onto column A then load 1.5 mL of the eluate from the SPE cartridge at 0.36 mL/min onto column A. Wash to waste with 500 μL water, 500 μL MeOH, and 500 μL water then elute the contents of column A onto column B with mobile phase A. After 11 min remove column A from the circuit and elute column B with a linear gradient of A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 10 min, re-equilibrate to 100:0. Monitor the effluent from column B. (Buffer A was 100 mM KH_2PO_4 containing 3 g/L pentanesulfonic acid. Succinate buffer was 60 g succinic acid in 1 L water adjusted to pH 4.0 with 1 M NaOH. Buffer B was 37.2 g disodium EDTA and 3 g pentanesulfonic acid in 1 L succinate buffer.)

HPLC VARIABLES

Column: A 10 × 6 10 μm Anagel-TSK-Chelate-SPW (Anachem); B 5 × 3 5 μm Polymer Labs. PLRP-S + 150 × 4.6 5 μm Polymer Labs. PLRP-S
Mobile phase: A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid and 10 mM EDTA.)
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM

Retention time: 25
Limit of detection: 20 μg/kg

OTHER SUBSTANCES

Extracted: demeclocycline, oxytetracycline, tetracycline

KEY WORDS

SPE; sheep; cattle; liver; kidney; column-switching

REFERENCE

Stubbings,G.; Tarbin,J.A.; Shearer,G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues, *J.Chromatogr.B*, **1996**, *679*, 137–145.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with saturated aqueous disodium EDTA. Blend 5 g tissue with two 20 mL portions and one 10 mL portion of 100 mM pH 4.0 disodium EDTA-McIlvaine buffer at high speed, centrifuge at 850 g for 5 min each time. Combine the supernatants, centrifuge at 850 g for 15 min, filter. Add the filtrate to the SPE cartridge, wash with 20 mL water, air-dry by aspiration for 5 min, elute with 10 mL ethyl acetate followed by 20 mL MeOH:ethyl acetate 5:95, evaporate the eluate to dryness under reduced pressure at 30°, dissolve the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK Gel Super Octyl (Tosoh)
Mobile phase: MeCN:0.05% aqueous trifluoroacetic acid 20:80
Flow rate: 0.5
Injection volume: 50
Detector: MS, Finnigan MAT TSQ 7000 Triple-Stage Quadrupole, electrospray voltage 4.5 kV, gas sheath flow 483 kPa nitrogen, collision gas argon, collision offset -25 V, m/z 479

CHROMATOGRAM
Retention time: 8.3

OTHER SUBSTANCES
Extracted: doxycycline, oxytetracycline, tetracycline

KEY WORDS
cow; SPE; kidney; liver; muscle

REFERENCE
Oka,H.; Ikai,Y.; Ito,Y.; Hayakawa,J.; Harada,K.-.; Suzuki,M.; Odani,H.; Maeda,K. Improvement of chemical analysis of antibiotics. XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *693*, 337–344.

SAMPLE
Matrix: tissue
Sample preparation: Prepare an affinity column by filling a 10 mL column with 5 mL chelating Sepharose, allow to settle, wash with 20 mL 0.5% copper(II) sulfate solution, eliminate air bubbles by agitation, wash with 15 mL 50 mM pH 4 succinate buffer, do not allow to dry. Condition an Analytichem Bond Elut C18 SPE cartridge with 10 mL MeOH and 10 mL water, do not allow to dry. Homogenize 4 g minced kidney with 40 mL 50 mM pH 4 succinate buffer, sonicate for 10 min, centrifuge at 9000 rpm for 10 min, filter the supernatant through paper, repeat the extraction. Combine the supernatants and pass them through the affinity column at 5-7 mL/min, wash with 10 mL water, wash with 30 mL MeOH, wash with 20 mL water, elute with 50 mL 50 mM pH 4 succinate buffer containing 3.7% Titriplex III (ethylenedinitrilotetracacetic acid, disodium salt dihydrate). Add the eluate to the SPE cartridge at 5-7 mL/min, wash with 10 mL water, dry with air aspiration for 10 min, elute with 5 mL MeOH:MeCN 1:1, evaporate the eluate at 40° under a stream of nitrogen, dissolve the residue in 500 µL mobile phase, inject an aliquot. Protect from light through process. (The affinity columns may be re-used up to 15 times by washing with 20 mL water then 20 mL EtOH:water 20:80 then conditioning as described above.)

HPLC VARIABLES
Guard column: Perisorb RP-8
Column: two 300 × 100 5 µm Chromspher C8 columns (cat. no. 28262) in series
Mobile phase: MeCN:10 mM pH 2 oxalic acid 20:80
Flow rate: 0.8
Detector: UV 365

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 30 ng/g

OTHER SUBSTANCES
Simultaneous: tetracycline, oxytetracycline, demethylchlortetracycline, methacycline, doxycycline

KEY WORDS
kidney; SPE

REFERENCE
Degroodt,J.M.; Wyhowski de Bukanski,B.; Srebrnik,S. Multiresidue analysis of tetracyclines in kidney by HPLC and photodiode array detection, *J.Liq.Chromatogr.*, **1993**, *16*, 3515–3529.

SAMPLE
Matrix: tissue

Sample preparation: Condition a 500 mg Separcol SI C18 SPE cartridge (Anapron) with 2 mL MeOH and 4 mL buffer. Homogenize 5 g muscle with 20 mL buffer and 3 mL n-hexane:dichloromethane 1:3 at 4°, centrifuge at 2400 g at 4° for 30 min, remove the supernatant, repeat homogenization with 10 mL buffer. Combine the supernatants, slowly add with constant stirring a volume of 1 g/mL trichloroacetic acid in water equal to 10% of the supernatant volume, stir for another min, keep in ice for 15 min, filter through paper, add to SPE cartridge at no more than 10 mL/min, wash with 2 mL water, elute with 4 mL 10 mM oxalic acid in MeOH, inject a 10 µL aliquot. (Buffer was 15 g $Na_2HPO_4.2H_2O$ + 13 g citric acid monohydrate + 3.72 g EDTA in 1 L water, pH 4.)

HPLC VARIABLES

Guard column: 5 µm LiChrospher 100 RP-18 guard column
Column: 250 × 4 5 µm HP Spherisorb ODS 2
Mobile phase: MeOH:MeCN:10 mM aqueous oxalic acid 20:35:45
Flow rate: 1
Injection volume: 10
Detector: UV 360

CHROMATOGRAM

Retention time: 8.5
Limit of detection: 50 ng/g

OTHER SUBSTANCES

Simultaneous: tetracycline, oxytetracycline

KEY WORDS

muscle; cow; pig; SPE

REFERENCE

Sokol,J.; Matisova,E. Determination of tetracycline antibiotics in animal tissues of food-producing animals by high-performance liquid chromatography using solid-phase extraction, *J.Chromatogr.A*, **1994**, *669*, 75–80.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut cyclohexyl (CH) SPE cartridge with 10 mL MeOH and 10 mL water. Powder (domestic food blender) frozen kidney or muscle. Homogenize (Silverson Machines) 5 g powdered tissue and 45 mL 100 mM glycine in 1 M HCl for 1 min, add 5 g ammonium sulfate, shake for 30 s, let stand for 10 min, centrifuge at 2000 rpm for 15 min, filter (glass wool) the supernatant, repeat the extraction with 50 mL 100 mM glycine in 1 M HCl. Combine the filtrates and centrifuge an aliquot at 2200 rpm for 10 min, add a 20 mL aliquot of the supernatant to the SPE cartridge, wash with 10 mL water, elute with 7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 65°, reconstitute the residue in 500 µL MeCN:20 mM oxalic acid 20:80, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: Chromspher C8 (Chrompack)
Column: 200 × 3 5 µm Chromspher C8 glass column (Chrompack)
Mobile phase: Gradient. A was MeCN. B was MeCN:20 mM oxalic acid 10:90. A:B from 10:90 to 20:80 over 2 min, maintain at 20:80 for 8 min, to 25:75 over 1 min, maintain at 25:75 for 9 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 0.4
Injection volume: 50
Detector: F ex 390 em 490 following post-column reaction. The column effluent mixed with 750 mM aluminum chloride (degas by sonication, store in a brown bottle) pumped at 0.6 mL/min and flowed through a 13.7 m × 0.3 mm i.d. PTFE column at 60° to the detector.

CHROMATOGRAM

Retention time: 20.4
Limit of detection: 70 ng/g (kidney), 30 ng/g (muscle)

OTHER SUBSTANCES

Extracted: oxytetracycline, tetracycline

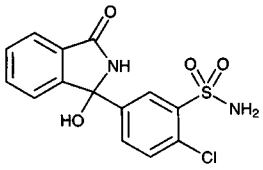
KEY WORDS

pig; cow; poultry; kidney; muscle; SPE; post-column reaction; complexation

REFERENCE

McCracken,R.J.; Blanchflower,W.J.; Haggan,S.A.; Kennedy,D.G. Simultaneous determination of oxytetracycline, tetracycline and chlortetracycline in animal tissues using liquid chromatography, post-column derivatization with aluminium, and fluorescence detection, *Analyst*, **1995**, *120*, 1763–1766.

Chlorthalidone



Molecular formula: $C_{14}H_{11}ClN_2O_4S$
Molecular weight: 338.77
CAS Registry No.: 77-36-1
Merck Index: 2246
Lednicer No.: 1 322

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 250 ng xipamide, mix for 10 s, add 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 µL mobile phase , mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 µm LiChrospher 100 RP-18
Column: 250 × 4 5 µm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: UV 225

CHROMATOGRAM

Retention time: 6.05
Internal standard: xipamide (8.58)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: albuterol, atenolol

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE

Matrix: formulations
Sample preparation: Grind 2 tablets, sonicate in 15 mL water for 15 min, shake vigorously for 30 min, add 25 mL MeOH, shake 1 h, dilute to 50 mL with MeOH, mix, centrifuge, filter (1 µm or smaller), inject 25 µL aliquot

HPLC VARIABLES

Column: 250 × 4.6 trimethylsilyl chloride bonded to 5-6 µm spherical silica
Mobile phase: MeOH:buffer 65:35 (Buffer was 2.2 mM KH_2PO_4 + 16 mM Na_2HPO_4, pH 7.9.)
Flow rate: 1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: clonidine

KEY WORDS
tablets

REFERENCE
Walters,S.M.; Stonys,D.B. Determination of chlorthalidone and clonidine hydrochloride in tablets by HPLC, *J.Chromatogr.Sci.*, **1983**, *21*, 43–45.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ChiraDex, LichroCART
Mobile phase: MeOH:water 20:80 (A) or MeOH:pH 4.1 triethylammonium acetate buffer 2:98 (B)
Column temperature: 6 (A), 45 (B)
Flow rate: 1 (A) or 0.8 (B)
Detector: UV 220

CHROMATOGRAM
Retention time: 23.51 (enantiomer I, A), 37.60 (enantiomer II, A), 15.44 (enantiomer I, B), 20.67 (enantiomer II, B)

KEY WORDS
chiral

REFERENCE
Cabrera,K.; Jung,M.; Fluck,M.; Schurig,V. Determination of enantiomerization barriers by computer simulation of experimental elution profiles obtained by high-performance liquid chromatography on a chiral stationary phase, *J.Chromatogr.A*, **1996**, *731*, 315–321.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.14 (A), 4.03 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in MeOH:water 80:20, inject a 6 μL aliquot.

HPLC VARIABLES

Guard column: 5 × 4 10 μm LiChrosorb RP-8

Column: 100 × 4.6 5 μm Spheri RP-18 (Brownlee)

Mobile phase: MeOH:water 80:20 containing 2 g/L lithium perchlorate

Flow rate: 0.5

Injection volume: 6

Detector: E, ESA Model 5100A Coulochem, model 5020 guard cell +950 mV, Model 5010 analytical cell + 400 mV, palladium reference electrode, following post-column photolysis. The effluent from the column flowed through a 20 m × 0.3 mm coil of PTFE tubing and was irradiated at 254 nm with a Sylvania GTE 8 W low-pressure lamp.

CHROMATOGRAM

Limit of detection: 267 ng/mL

OTHER SUBSTANCES
Also analyzed: bendroflumethiazide, butizide, ethacrynic acid, furosemide, hydrochlorothiazide

KEY WORDS
post-column reaction

REFERENCE
Macher,M.; Wintersteiger,R. Improved electrochemical detection of diuretics in high-performance liquid chromatographic analysis by postcolumn on-line photolysis, *J.Chromatogr.A*, **1995**, *709*, 257–264.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of an 8 μg/mL solution.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 100 RP-18
Mobile phase: EtOH:buffer 20:80 containing 25 mM β-cyclodextrin substituted with 2-hydroxy-3-trimethylammoniumpropyl groups (Roquette Frères, Lestrem, France) (Buffer was 0.8% triethylamine adjusted to pH 4.1 with acetic acid.)
Column temperature: 22.5
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11.26 (+), 12.31 (-)

KEY WORDS
chiral

REFERENCE
Roussel,C.; Favrou,A. Cationic β-cyclodextrin: a new versatile chiral additive for separation of drug enantiomers by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 67–74.

SAMPLE
Matrix: urine
Sample preparation: Inject 50 μL untreated urine onto column A and elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B. After another 5 min re-equilibrate column A with mobile phase A and column B with mobile phase B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μmHypersil ODS C18; B 250 × 4 5 μm LiChroCART ChiraDex (Merck)
Mobile phase: A Water; B MeOH:50 mM pH 4 acetate buffer 40:60 (Prepare buffer by adding 500 μL propylamine hydrochloride to 500 mL water, dissolve 2 g sodium acetate, adjust pH to 4 with glacial acetic acid.) (Mobile phase B is also given as MeCN:buffer 40:60 and MeOH:buffer 60:40.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 7.8, 8.2 (enantiomers)
Limit of detection: 20ng/mL
Limit of quantitation: 250 ng/mL

KEY WORDS
chiral; direct injection; column-switching; pharmacokinetics

REFERENCE
Herráez-Hernández,R.; Campíns-Falco,P.; Sevillano-Cabeza,A. Application of column switching in high-performance liquid chromatographic analysis of chlorthalidone enantiomers in untreated urine, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 403–414.

SAMPLE
Matrix: urine
Sample preparation: Add 400 μL 3 μg/mL IS in water to 600 μL urine, inject a 50 μL aliquot onto column A at 1 mL/min. Elute to waste with 2 mL water, after 2 min backflush the contents of column A onto column B with mobile phase, after 30 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with MeCN:water 95:5 and equilibrate it with water.

HPLC VARIABLES
Column: A 12.5 × 4 5 μm Stable Bond-CN (Zorbax); B 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 20:80
Flow rate: 2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 9.8
Internal standard: 2,7-dihydroxynaphthalene (11.2)
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: 2-(3-aminosulfonyl-4-chlorbenzoyl) benzoic acid, caffeine
Noninterfering: methyldopa, salicylic acid
Interfering: naproxen

KEY WORDS
column-switching; pharmacokinetics

REFERENCE
Salado,S.C.; Vera-Avila,L.E. On-line solid-phase extraction and high-performance liquid chromatographic determination of chlorthalidone in urine, *J.Chromatogr.B*, **1997**, *690*, 195–202.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM

Retention time: 4.1
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES

Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, methyclothiazide, clopamide, furosemide, metolazone, mefruside, bendroflumethiazide, cyclopenthiazide, bumetanide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE

Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE

Matrix: urine
Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 9.0 (A), 9.8 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE
Matrix: urine
Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)
Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 4.5:10.5:85:0.5
Flow rate: 0.8 or 1
Injection volume: 10-20
Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 100 ng (by MS)

OTHER SUBSTANCES
Extracted: amiloride, triamterene, furosemide, benzthiazide, bendroflumethiazide

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Direct injection.

HPLC VARIABLES
Guard column: 35 × 4 5 μm Spherisorb ODS-2
Column: 120 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:50 mM sodium dodecyl sulfate 5:95
Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 224

CHROMATOGRAM
Retention time: 8.8
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: bendroflumethiazide

REFERENCE
Bonet Domingo,E.; Medina Hernández,M.J.; Ramis Ramos,G.; Garcia Alvarez-Coque,M.C. High-performance liquid chromatographic determination of diuretics in urine by micellar liquid chromatography, *J.Chromatogr.*, **1992**, *582*, 189–194.

SAMPLE
Matrix: urine
Sample preparation: Condition a 1 mL 100 mg Bond-Elut C8 SPE cartridge with 500 μL MeOH and 500 μL water. 2 mL urine + 300 μL 1 μg/mL triamterene in MeOH, add to

the SPE cartridge, wash with 500 μL water, elute with 500 μL MeOH, filter (0.45 μm) the eluate, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm HP-LiChrospher 100 RP 18
Mobile phase: Gradient. MeCN:buffer from 0:100 to 30:70 over 5 min, maintain at 30:70. (Buffer was 3.45 g $NaH_2PO_4.H_2O$ + 700 μL propylamine hydrochloride in 500 mL water, adjust pH to 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 6.1
Internal standard: triamterene (3.8)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES
Simultaneous: atenolol, oxprenolol, reserpine, spironolactone
Noninterfering: metabolites

KEY WORDS
SPE

REFERENCE
Campíns-Falcó,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Simple and sensitive reversed-phase liquid chromatographic assay for analysis of chlorthalidone in urine, *J.Liq.Chromatogr.*, **1993**, *16*, 2571–2581.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 13.7
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 4.7

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, cyclothiazide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

Interfering: morazone, diclofenamide

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18

Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 7.5

Limit of detection: 4 ng/mL.

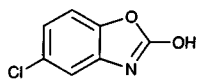
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Chlorzoxazone



Molecular formula: $C_7H_4ClNO_2$
Molecular weight: 169.57
CAS Registry No.: 95-25-0
Merck Index: 2249
Lednicer No.: 1 323

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 1 mL 200 mM pH 4.75 sodium acetate buffer + 500 μL 0.2% sodium chloride containing 1000 U β-glucuronidase, vortex, incubate at 37° for 3 h. Add 100 μL 20 μg/mL IS, 400 μL 10% perchloric acid and 4 mL ethyl acetate, vortex for 5 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it under a gentle stream of nitrogen at 40°. Reconstitute the residue in 50 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS (B)
Mobile phase: MeCN:0.15% pH 5.0 ammonium acetate 10:90
Flow rate: 0.3
Injection volume: 10
Detector: UV 287

CHROMATOGRAM

Retention time: 7 (A), 11 (B)
Internal standard: 5-chloro-2-methylbenzoxazole (12 (A); 19 (B))
Limit of quantitation: 10 ng/mL (A)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; pharmacokinetics

REFERENCE

Tanaka,E. Simultaneous determination of chlorzoxazone, indicator of CYP2E1, and its metabolite in human serum using a new reversed-phase chromatographic column of 2-μm porous microspherical silica-gel, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 899–904.

SAMPLE

Matrix: blood
Sample preparation: Evaporate 100 μL 100 μg/mL phenacetin in MeOH into the bottom of a tube with a stream of nitrogen, add 1 mL plasma, vortex for 1 min, add 1 mL 50 mM sulfuric acid, add four 125 mg portions of ammonium sulfate with vortexing and heating on a steam bath after each addition, add 5 mL ether, vortex, centrifuge at 5000 rpm for 5 min, freeze in dry ice/acetone. Remove the organic layer and filter (0.5 μm) it, evaporate to dryness under a stream of nitrogen on a steam bath, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60
Flow rate: 2
Injection volume: 20

Detector: UV 280

CHROMATOGRAM
Retention time: 8.5
Internal standard: phenacetin (6)
Limit of detection: 80 ng

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen

KEY WORDS
plasma

REFERENCE
Honigberg,I.L.; Stewart,J.T.; Coldren,J.W. Liquid chromatography in pharmaceutical analysis X: Determination of chlorzoxazone and hydroxy metabolite in plasma, *J.Pharm.Sci.*, **1979**, *68*, 253–255.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with one volume of MeOH and one volume of 50 μL/L glacial acetic acid in water. 500 μL Plasma + 10 μL 40 μg/mL 5-fluorobenzoxazolone in MeOH + 1 mL 200 mM pH 4.75 sodium acetate buffer + 500 μL 0.2% NaCl containing 1000 U β-glucuronidase (Type G0751, Sigma), vortex, heat at 37° for 3 h, add to SPE cartridge, wash with 1 mL 50 μL/L glacial acetic acid in water, elute with two 500 μL aliquots of MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeCN:100 mM ammonium acetate 40:60, vortex, centrifuge at 13600 g for 1 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:THF:100 mM pH 7.0 ammonium acetate 22.5:5.5:72
Flow rate: 1
Injection volume: 50
Detector: UV 283

CHROMATOGRAM
Retention time: 17.65
Internal standard: 5-fluorobenzoxazolone (10.53)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Stiff,D.D.; Frye,R.F.; Branch,R.A. Sensitive high-performance liquid chromatographic determination of chlorzoxazone and 6-hydroxychlorzoxazone in plasma, *J.Chromatogr.*, **1993**, *613*, 127–131.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Whole blood or plasma + 25 μL 250 μg/mL p-acetophenetide, vortex, add 2 mL MeCN, vortex, centrifuge at high speed for 5 min. Remove the MeCN

layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Econosil C18
Mobile phase: MeOH:water 40:60
Flow rate: 2
Injection volume: 50
Detector: UV (wavelength not given)

CHROMATOGRAM
Internal standard: p-acetophenetide
Limit of detection: 4 ng/mL

KEY WORDS
pharmacokinetics; whole blood; plasma

REFERENCE
de Vries,J.D.; Salphati,L.; Horie,S.; Becker,C.E.; Hoener,B.A. Variability in the disposition of chlorzoxazone, *Biopharm.Drug Dispos.*, **1994**, *15*, 587–597.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 1 mL 200 mM pH 4.75 sodium acetate buffer + 1000 U β-glucuronidase in 500 μL 0.2% NaCl, vortex, incubate at 37° for 3 h. Add 5 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 10 min. Evaporate the organic layer under a stream of nitrogen at 40°. Reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot. Urine. Dilute sample 1:1 (v/v) with water. 500 μL Diluted urine + 1 mL 200 mM pH 4.75 sodium acetate buffer + 1000 U β-glucuronidase in 500 μL 0.2% NaCl, vortex, incubate at 37° for 3 h. Add 5 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 10 min. Evaporate the organic layer under a stream of nitrogen at 40°. Reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18 (Alltech)
Column: 300 × 3.9 10 μm Alphabond C18 (Alltech) (plasma) or 300 × 3.9 10 μm μBondapak C18 (urine)
Mobile phase: MeCN:THF:100 mM ammonium acetate 22.5:5.5:72
Flow rate: 1.0
Injection volume: 50
Detector: UV 283

CHROMATOGRAM
Retention time: 17.5 (plasma)
Internal standard: 3-aminophenyl sulfone (plasma, 12.1), phenacetin (urine, 10.9)
Limit of quantitation: 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Frye,R.F.; Stiff,D.D. Determination of chlorzoxazone and 6-hydroxychlorzoxazone in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *686*, 291–296.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 1:100. 500 μL Serum or diluted urine + 500 μL 2 M pH 4.5 acetate buffer + 20 μL H. pomatia juice, heat at 37° overnight, add 4 mL 600 mM perchloric acid, centrifuge at 3500 g for 10 min. Remove the supernatant and add it to 4 mL ethyl acetate, shake for 10 min, centrifuge at 4° for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 20 μL aliquot. (H. pomatia juice from IBF Biotechnics contained 100000 Fishman U/mL β-glucuronidase and 1000000 Roy U/mL sulfatase.)

HPLC VARIABLES
Column: 250 × 4.6 5μm Nucleosil ODS
Mobile phase: MeCN:0.5% acetic acid 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 287

CHROMATOGRAM
Retention time: 17
Limit of detection: 50 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics

REFERENCE
Lucas,D.; Berthou,F.; Girre,C.; Poitrenaud,F.; Ménez,J.-F. High-performance liquid chromatographic determination of chlorzoxazone and 6-hydroxychlorzoxazone in serum: a tool for indirect evaluation of cytochrome P4502E1 activity in humans, *J.Chromatogr.*, **1993**, *622*, 79–86.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 200 μL 1.5 μg/mL phenacetin in MeCN to 400 μL microsomal incubation, vortex for 30 s, centrifuge at 10000 g for 4 min. Add 2 mL diethyl ether to the supernatant, vortex for 30 s, centrifuge at 2000 g for 1 min, dry the ether layer under vacuum, reconstitute the residue with 150 μL mobile phase, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Brownlee Spheri-5 C8
Mobile phase: MeCN:0.5% phosphoric acid 30:70
Flow rate: 1
Injection volume: 100
Detector: UV 287

CHROMATOGRAM
Retention time: 6.4
Internal standard: phenacetin (4.3)

OTHER SUBSTANCES
Extracted: 6-hydroxychlorzoxazone

KEY WORDS
liver; rat

REFERENCE

Chittur,S.V.; Tracy,T.S. Rapid and sensitive high-performance liquid chromatographic assay for 6-hydroxychlorzoxazone and chlorzoxazone in liver microsomes, *J.Chromatogr.B*, **1997**, *693*, 479–483.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 5 mL ethyl acetate + umbelliferone + 50 μL 42.5% phosphoric acid, vortex, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm C18 (Supelco)
Mobile phase: Gradient. MeCN:0.25% acetic acid 21:79 for 11 min, to 95:5 over 7 min.
Flow rate: 1
Detector: UV 296

CHROMATOGRAM

Retention time: 11.54
Internal standard: umbelliferone (4.76)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver

REFERENCE

Jayyosi,Z.; Knoble,D.; Muc,M.; Erick,J.; Thomas,P.E.; Kelley,M. Cytochrome P-450 2E1 is not the sole catalyst of chlorzoxazone hydroxylation in rat liver microsomes, *J.Pharmacol.Exp.Ther.*, **1995**, *273*, 1156–1161.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.01 (A), 5.87 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine,

doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

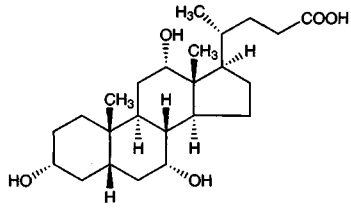
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Cholic acid



Molecular formula: $C_{24}H_{40}O_5$
Molecular weight: 408.58
CAS Registry No.: 81-25-4
Merck Index: 2258

SAMPLE

Matrix: bile, blood

Sample preparation: Serum. 100-200 µL Serum + 1 mL MeOH, mix, sonicate for 15 min. Remove a 600 µL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute with 1 mL 50 mM pH 7.0 phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 µL aliquot and add it to 50 µL 100 µM lauric acid in MeOH, add 50 µL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 µL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 µL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 µL aliquot. Bile. Mix 10 µL bile with 10 mL 50 mM pH 7.0 phosphate buffer, add a 1 mL aliquot to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 µL aliquot and add it to 50 µL 100 µM lauric acid in MeOH, add 50 µL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 µL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 µL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 µL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 µm Model RCM-100 Radial-Pak A (Waters)
Mobile phase: Gradient. MeCN:MeOH:water 100:50:40 for 30 min then 100:50:20 (step gradient).
Flow rate: 2
Injection volume: 8
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 32
Internal standard: lauric acid (56)
Limit of detection: 10 pmole
Limit of quantitation: 50 pmole

OTHER SUBSTANCES

Extracted: chenodiol, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; serum; SPE

REFERENCE

Kamada,S.; Maeda,M.; Tsuji,A. Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, *J.Chromatogr.*, **1983**, *272*, 29–41.

SAMPLE

Matrix: solutions

Sample preparation: Mix an aliquot of solution (or hydrolyzed bile) with a 50% molar excess of triethylamine in MeCN, warm briefly, add a 50% molar excess of 100 mM 2-bromoacetophenone in MeCN, heat at 80-90° for 45-60 min, evaporate to dryness, reconstitute with dioxane (Caution! Dioxane is a carcinogen!), filter (0.47 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10/25 ODS
Mobile phase: Gradient. n-Heptane:dioxane 90:10 for 3 min then n-heptane:dioxane:isopropanol 70:25:5 (step gradient). (Caution! Dioxane is a carcinogen!)
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 26
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, deoxycholic acid, hyodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS
derivatization

REFERENCE
Stellaard,F.; Hachey,D.L.; Klein,P.D. Separation of bile acids as their phenacyl esters by high-pressure liquid chromatography, *Anal.Biochem.*, **1978**, *87*, 359–366.

SAMPLE
Matrix: solutions
Sample preparation: Treat a solution in MeOH with a slight excess of tetramethylammonium hydroxide in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with MeCN, add a 2-10 fold excess of 9-(chloromethyl)anthracene in cyclohexane, heat at 75° for 15 min, very dilute solutions may require longer times), dilute with MeCN, inject an aliquot.

HPLC VARIABLES
Column: 300 mm long "Fatty Acid" reversed-phase (Waters)
Mobile phase: MeOH:water 88:12 (A) or 82:18 (B)
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: chenodiol, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid

KEY WORDS
derivatization

REFERENCE
Korte,W.D. 9-(Chloromethyl)anthracene: a useful derivatizing reagent for enhanced ultraviolet and fluorescence detection of carboxylic acids with liquid chromatography, *J.Chromatogr.*, **1982**, *243*, 153–157.

SAMPLE
Matrix: solutions

Sample preparation: Mix 200 μL of a solution of bile acids with 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, add 300 μL 10 mM tetrakis(decyl)ammonium bromide in 100 mM pH 7.0 phosphate buffer, heat at 40° for with sonication 10 min, add 300 μL 5.1 μM IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 Ultracarb 5 ODS
Mobile phase: Gradient. A was water. B was MeCN:MeOH 60:40. A:B 55:45 for 20 min, to 30:70 over 10 min, maintain at 30:70 for 25 min, return to initial conditions over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 16
Internal standard: 6-methoxynaphthacyl ester of lauric acid (36)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Simultaneous: chenodiol, deoxycholic acid, lithocholic acid, ursodiol

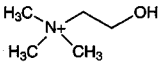
KEY WORDS

derivatization

REFERENCE

Gatti,R.; Roda,A.; Cerre,C.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum, *Biomed.Chromatogr.*, **1997**, *11*, 11–15.

Choline



Molecular formula: $C_5H_{14}NO$

Molecular weight: 107.17

CAS Registry No.: 62-49-7, 67-48-1 (chloride), 4201-78-9 (dehydrocholate), 77-91-8 (dihydrogen citrate), 2016-36-6 (salicylate), 4499-40-5 (theophyllinate), 87-67-2 (bitartrate), 507-30-2 (gluconate)

Merck Index: 2259

SAMPLE

Matrix: CSF, dialysate, tissue

Sample preparation: Dialysate. Inject dialysate directly. Tissue. Homogenize brain tissue with 10 volumes 100 mM perchloric acid (Potter-Elvehjem), let stand on ice for 15 min, centrifuge at 4000 g for 15 min, inject a 0.5 μL aliquot. CSF. Deproteinize by passing through a 0.02 μm Anatop 10 syringe filter (Alltech), inject a 0.5 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 1 PEEK column packed with Aminex A-9 (Bio-Rad)

Mobile phase: 200 mM pH 8.0 K/Na 3/1 phosphate buffer containing 5 mM NaCl and 0.1% Kathon CG

Column temperature: 25

Flow rate: 0.06 (obtained with a flow splitter)

Injection volume: 0.5

Detector: E, AMOR (Spark Holland), platinum working electrode + 250 mV, carbon composite auxiliary electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent flowed through a reactor which had 4 U acetylcholine esterase (EC 3.1.1.7 type VI-S from electric eel, 260 IU/mg) and 4 U choline oxidase (EC 1.1.3.17 from Alcaligenes sp., 12.7 IU/mg) enclosed between two 0.01 μm cellulose nitrate filters (Sartorius) (construction details given) to the detector.

CHROMATOGRAM

Retention time: 8

Limit of detection: 10 fmole

OTHER SUBSTANCES

Extracted: acetylcholine

KEY WORDS

use metal-free tubing and connectors; solvent reservoir; column; reactor; and detector maintained at 25; rat; brain; human; post-column reaction; immobilized enzyme reactor

REFERENCE

Flentge,F.; Venema,K.; Koch,T.; Korf,J. An enzyme-reactor for electrochemical monitoring of choline and acetylcholine: applications in high-performance liquid chromatography, brain tissue, microdialysis and cerebrospinal fluid, *Anal.Biochem.*, **1992**, *204*, 305–310.

SAMPLE

Matrix: blood

Sample preparation: 250 μL Plasma + 25 μL 0.41 mM IS + 750 μL ice-cold 1 M formic acid in acetone, mix, centrifuge for 15 min. Remove the supernatant and add it to the SPE column, wash with 1 mL 100 mM pH 4.0 ammonium acetate, elute with 1 mL 2 M NaCl in MeOH:water 50:50, force out all liquid under pressure. Evaporate the eluate under a stream of nitrogen and keep under vacuum for 1 h, add 1 mL MeCN to the residue, mix, centrifuge, remove the supernatant, repeat the extraction. Combine the supernatants and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 300 μL freshly prepared 21.7 mM 3,5-dinitrobenzoyl chloride in pyridine (dry

pyridine over KOH), heat at 105° for 1 h, evaporate the pyridine under a stream of nitrogen, extract the residue with 300 μL water then with 200 μL water, combine the extracts, filter (0.3 μm), inject a 100 μL aliquot. (Preparation of SPE column. Let 300 mg AG 50W-X12 cation-exchange resin sit overnight in 1 mL 100 mM pH 4.0 ammonium acetate. Add the mixture to a Pasteur pipette with a glass wool plug, wash column with 1 mL 2 M NaCl in MeOH:water 50:50, activate column with 1 mL 100 mM pH 4.0 ammonium acetate.)

HPLC VARIABLES

Column: Two 300 × 4 10 μm μBondapak C18 in series
Mobile phase: MeCN:water 50:50 containing 5 mM sodium dodecyl sulfate and 0.1% acetic acid
Flow rate: 2-2.3
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 13
Internal standard: 3-hydroxy-N,N,N-trimethylpropanaminium iodide (15) (Prepare by adding 18.5 g iodomethane to 10 g 3-hydroxy-N,N-dimethylaminopropane in 24 mL EtOH, stir, filter, add cold diethyl ether to the filtrate, filter. Combine precipitates, recrystallize from EtOH/diethyl ether, mp 203-4°)
Limit of quantitation: 1 nmole/mL

KEY WORDS

plasma; SPE; derivatization

REFERENCE

Buchanan,D.N.; Fucek,F.R.; Domino,E.F. Paired-ion high-performance liquid chromatographic assay for plasma choline, *J.Chromatogr.*, **1980**, *181*, 329–335.

SAMPLE

Matrix: blood
Sample preparation: Add neostigmine and ethylhomocholine to plasma or red blood cells. 150 μL Plasma or red blood cells + 1 mL 400 mM perchloric acid, let stand at 0-4° for 30 min, centrifuge at 5500 g for 1 min. Remove a 750 μL aliquot of the supernatant and add it to 36 μL 10 M potassium acetate, let stand at 0-4° for 5 min, centrifuge at 5500 g for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 1.9 Chrompack reversed phase
Column: 100 × 3 Chromspher 5 C18 (Chrompack) (Prepare column by washing with MeOH, MeOH:water 50:50, water, and 5 mg/mL sodium laurylsulfate in water (each wash 20 min at 1 mL/min). Thoroughly wash pump with water (column off line), wash column with water for 5 min and mobile phase for 1 h. Column should be disconnected from the precolumn, reactor, and detector. [Chromatographia, 1987,24,827].)
Mobile phase: 200 mM pH 8.0 potassium phosphate buffer containing 5 mM KCl
Flow rate: 0.6
Injection volume: 100
Detector: E, Spark Holland Amor, Pt working electrode +500 mV, carbon composite auxiliary electrode, Ag/AgCl reference electrode following an enzyme reactor. (Reactor was a 10 × 2.1 Hypersil APS-2 column, activate with glutaraldehyde, equilibrate with mobile phase for 20 min. Inject 80 U acetylcholine esterase (EC 3.1.1.7, type VI-S from electric eel, 260 IU/mg) and 40 U choline oxidase (EC 1.1.3.17 from Alcaligenes sp., 12.7 IU/mg) in 500 μL mobile phase onto the reactor and pump through at 0.05 mL/min for 20 min with mobile phase. [Chromatographia, 1987,24,827])

CHROMATOGRAM
Retention time: 2.5
Internal standard: ethylhomocholine (5)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: acetylcholine

KEY WORDS
plasma; red blood cells; human; mouse; post-column reaction; immobilized enzyme reactor

REFERENCE
Damsma,G.; Flentge,F. Liquid chromatography with electrochemical detection for the determination of choline and acetylcholine in plasma and red blood cells. Failure to detect acetylcholine in blood of humans and mice, *J.Chromatogr.*, **1988**, *428*, 1–8.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 2 mL 300 ng/mL ethylhomocholine in MeCN, vortex for 1 min, centrifuge at 2000 g for 15 min. Remove 1.5 mL of the supernatant and evaporate it to dryness under vacuum, reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Cyano Spheri-5
Mobile phase: 20 mM sodium hydrogen phosphate and 10 mM tetramethylammonium chloride adjusted to pH 7.1 with 65% phosphoric acid
Flow rate: 0.7
Injection volume: 50
Detector: E, ESA Coulochem II, Model 5040 Pt analytical cell + 300 mV, following an enzyme reactor. (The reactor was a 30 × 2.1 7 μm Aquapore AX-300 anion-exchange cartridge (Brownlee), inject slowly 50 μL 100 U/mL choline oxidase (EC 1.1.3.17, Alcaligenes) and catalase (EC 1.11.1.6) (Sigma), wash with mobile phase for several minutes before use, reload after 100 samples.)

CHROMATOGRAM
Retention time: 5
Internal standard: ethylhomocholine (8)
Limit of quantitation: 3.58 μM

KEY WORDS
pharmacokinetics; immobilized enzyme reactor; plasma; post-column reaction

REFERENCE
Fossati,T.; Colombo,M.; Castiglioni,C.; Abbiati,G. Determination of plasma choline by high-performance liquid chromatography with a postcolumn enzyme reactor and electrochemical detection, *J.Chromatogr.B*, **1994**, *656*, 59–64.

SAMPLE
Matrix: bulk
Sample preparation: Heat 500 nmole with 500 μL 1 mg/mL benoxaprofen chloride in MeCN or toluene at 60-80° for 1 h, inject a 10 μL aliquot. (Prepare benoxaprofen chloride as follows. Dissolve 600 mg benoxaprofen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 30 min, evaporate to dryness, recrystallize from dichloromethane (if necessary) to give benoxaprofen chloride (mp 91.5°).)

HPLC VARIABLES
Column: 120 × 4.6 5 μm LiChrosorb RP-8

Mobile phase: Acetone:10 mM sodium heptanesulfonate:phosphoric acid 60:40:0.15
Column temperature: 55
Flow rate: 2
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: scopolamine N-butylbromide

KEY WORDS
derivatization

REFERENCE
Spahn,H.; Weber,H.; Mutschler,E.; Möhrke,W. α-Alkyl-α-arylacetic acid derivatives as fluorescence markers for thin-layer chromatographic and high-performance liquid chromatographic assay of amines and alcohols, *J.Chromatogr.*, **1984**, *310*, 167–178.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dilute succinylcholine chloride bulk drug and formulation samples in MeCN:100 mM hexanesulfonic acid:water 5:20:75, inject an aliquot. (Prepare the diluent by diluting 100 mL 100 mM hexanesulfonic acid and 25 mL MeCN to 500 mL with water.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima C18
Mobile phase: Gradient. A was MeCN:5 mM hexanesulfonic acid:water 5:5:90. B was MeCN:water 50:50. A:B 100:0 for 13 min, from 100:0 to 0:100 in 2 min, maintain at 0:100 for 10 min, from 0:100 to 100:0 in 2 min, maintain at 100:0 for 8 min
Flow rate: 1
Injection volume: 50
Detector: Conductivity, Waters M-430 conductivity detector coupled with a Dionex CMMS-II cation micromembrane suppressor with suppression regenerant of 25 mM tetrabutylammonium hydroxide at 5 mL/min

CHROMATOGRAM
Retention time: 11
Limit of detection: 10 pmol

OTHER SUBSTANCES
Noninterfering: succinylcholine chloride

REFERENCE
Chen,S.; Soneji,V.; Webster,J. Determination of choline in pharmaceutical formulations by reversed-phase high-performance liquid chromatography and postcolumn suppression conductivity detection, *J.Chromatogr.A*, **1996**, *739*, 351–357.

SAMPLE
Matrix: dialysate
Sample preparation: Inject 10 μL of rat brain dialysate.

HPLC VARIABLES
Guard column: ACH-3-G guard cartridge (ESA)
Column: 150 × 3 5 μm ACH-3 polymeric reversed-phase column (ESA)

Mobile phase: 100 mM sodium phosphate + 0.5 mM tetramethylammonium chloride + 0.005% Reagent MB (a microbicide from ESA) + 2 mM octanesulfonic acid, final pH 8.0
Column temperature: 35
Flow rate: 0.35
Injection volume: 10
Detector: E, ESA Coulochem Model 5200A, Model 5040 analytical cell, palladium reference electrode, stainless steel counter electrode, platinum working electrode + 300 mV following a solid-phase reactor containing immobilized acetylcholinesterase and choline oxidase (reactor temp 35)

CHROMATOGRAM
Retention time: 4
Limit of detection: 2 μM

OTHER SUBSTANCES
Extracted: acetylcholine

KEY WORDS
brain; rat; post-column reaction; immobilized enzyme reactor

REFERENCE
Greaney,M.D.; Marshall,D.L.; Bailey,B.A.; Acworth,I.N. Improved method for the routine analysis of acetylcholine release in vivo: quantitation in the presence and absence of esterase inhibitor, *J.Chromatogr.*, **1993**, *622*, 125–135.

SAMPLE
Matrix: dialysate
Sample preparation: Inject 5 μL aliquots of the dialysate (Ringer's solution).

HPLC VARIABLES
Column: 530 × 1 cation exchange MF-8904 (Bioanalytical Systems)
Mobile phase: 50 mM Na_2HPO_4 containing 5 mL/L Kathon CG (Bioanalytical Systems CF-2150) (Mobile phase was only partially degassed; some oxygen is essential for the enzyme reactor.)
Flow rate: 0.14
Injection volume: 5
Detector: E, Bioanalytical Systems LC-4C, peroxidase-redox polymer coated glassy carbon electrode +100 mV (Anal.Chem. 1992, 64, 3084), Ag/AgCl reference electrode. The column effluent passed through a 50 × 1 immobilized-enzyme reactor containing acetylcholinesterase (EC 3.1.1.7) and choline oxidase (EC 1.1.3.17) (Bioanalytical Systems MF-8903) and flowed to the detector.

CHROMATOGRAM
Retention time: 24
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: acetylcholine

KEY WORDS
rat; post-column reaction; immobilized enzyme reactor

REFERENCE
Huang,T.; Yang,L.; Gitzen,J.; Kissinger,P.T.; Vreeke,M.; Heller,A. Detection of basal acetylcholine in rat brain microdialysate, *J.Chromatogr.B*, **1995**, *670*, 323–327.

SAMPLE
Matrix: formulations

Sample preparation: Make up the lyophilized preparation in sterile water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Mix (? g) sodium 1-heptanesulfonate (Waters PIC Reagent B-7) in 900 mL water, adjust pH to 4.0 with 6 M ammonium hydroxide, add 50 mL MeCN, make up to 1 L with water
Flow rate: 2
Injection volume: 50
Detector: RI

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: acetylcholine
Noninterfering: acetic acid, mannitol

REFERENCE
Tao,F.T.; Thurber,J.S.; Dye,D.M. High-performance liquid chromatographic determination of acetylcholine in a pharmaceutical preparation, *J.Pharm.Sci.*, **1984**, *73*, 1311–1313.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 0.26 silica A (Perkin-Elmer)
Mobile phase: MeOH:water:500 mM tetramethylammonium sulfate 65:25:10
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Simultaneous: methyl p-hydroxybenzoate, succinic acid, succinylcholine, succinylmonocholine

KEY WORDS
injections; saline

REFERENCE
Schmutz,C.W.; Mühlebach,S.F. Stability of succinylcholine chloride injection, *Am.J.Hosp.Pharm.*, **1991**, *48*, 501–506.

SAMPLE
Matrix: formulations
Sample preparation: Condition a Sep Pak C18 SPE cartridge with 2 mL MeOH and 2 mL water, pass the formulation through the SPE cartridge to remove fat-soluble vitamins, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 125 × 4 5 μm Nucleosil SA or 300 × 4.6 5 μm Zorbax SCX 300 Å

Mobile phase: EtOH:100 mM pH 5.0 ammonium acetate 20:80 containing 0.3 mM 3-hydroxytyramine hydrochloride (After use wash column with EtOH:water 20:80. Keep column in EtOH:water 70:30 when not in use.)
Column temperature: 40
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.3

KEY WORDS
indirect UV detection; SPE

REFERENCE
Leroy,P.; Barbaras,M.; Colin,J.L.; Nicolas,A. Ion-exchange liquid chromatography method with indirect UV detection for the assay of choline in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 581–588.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 4 mM solution in water, inject a 10 μL aliquot

HPLC VARIABLES
Column: μBondapak C18 Radial-Pak in a RCM-100 radial compression module
Mobile phase: Butanol:MeOH:acetic acid:water 8:4:2:86 containing 0.15 mM 1-phenethyl-2-picolinium bromide (Extract 10 mM 1-phenethyl-2-picolinium bromide stock solution with dichloromethane before use to remove UV-absorbing impurities.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: acetylcholine, butyrylcholine, propionylcholine

REFERENCE
Jones,R.S.; Stutte,C.A. Chromatographic analysis of choline and acetylcholine by UV visualization, *J.Chromatogr.*, **1985**, *319*, 454–460.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was hexane. B was ethyl acetate. C was 0.1% formic acid in MeCN. D was 0.1% formic acid in water. A:B:C:D 100:0:0:0 for 5 min, to 0:100:0:0 over 15 min, maintain at 0:100:0:0 for 5 min, to 0:0:100:0 over 5 min, maintain at 0:0:100:0 for 5 min; to 0:0:0:100 over 25 min, maintain at 0:0:0:100 for 5 min.
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM
Retention time: 55.04

OTHER SUBSTANCES

Simultaneous: acetylcholine, cholesterol, cortisone, dextrose, estradiol, glycine, phenylalanine, sodium, testosterone

REFERENCE

Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 Diol

Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN. B was 0.1% trifluoroacetic acid in water. A:B 90:10 for 1 min, to 70:30 over 17 min, to 0:100 over 2 min, maintain at 0:100 over 4.5 min

Flow rate: 0.9

Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM

Retention time: 5.08

OTHER SUBSTANCES

Simultaneous: acetylcholine, sodium, magnesium, calcium

REFERENCE

Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize brain tissue with 3 mL 400 mM perchloric acid containing 2 nmoles ethylhomocholine, centrifuge at 35000 g for 20 min, adjust pH of supernatant to about 4.2 with about 200 μL 7.5 M potassium acetate, centrifuge at 35000 g for 20 min. Add the supernatant to 100 μL 5 mM tetramethylammonium chloride, add 3 mL 2% ice-cold reineckate solution, let stand on ice for 1 h, centrifuge at 1000 g at 0° for 10 min. Remove the supernatant and dry the precipitate under vacuum overnight, add about 1 mL 5 mM silver tosylate in MeCN until the pink color disappears, centrifuge at 1000 g at 0° for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL 20 mM pH 3.5 citrate-phosphate buffer, inject an aliquot.

HPLC VARIABLES

Guard column: ODS-5 (Bio-Rad)

Column: 150 mm long Bio-Sil ODS-5S (Bio-Rad)

Mobile phase: Buffer (Buffer was 10 mM sodium acetate buffered to pH 5 with 20 mM citric acid, containing 4.5 mg/L sodium octyl sulfate and 1.2 mM tetramethylammonium chloride.)

Flow rate: 0.8

Injection volume: 20

Detector: E, Bio Analytical Systems LC-4A, Pt electrode +0.5 V, Ag/AgCl reference electrode following post-column reaction detection. The column effluent mixed with 1 U/mL choline oxidase and 2 U/mL acetylcholinesterase in 200 mM pH 8.5 phosphate buffer pumped at 0.5 mL/min, the mixture flowed through a 30 m × 0.3 mm i.d. PTFE tube (2.5 min) to the detector

CHROMATOGRAM
Retention time: 3.6
Internal standard: ethylhomocholine (7.2)
Limit of detection: 1 pmole

OTHER SUBSTANCES
Extracted: acetylcholine

KEY WORDS
post-column reaction; rat; brain; immobilized enzyme reactor

REFERENCE
Potter,P.E.; Meek,J.L.; Neff,N.H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection, *J.Neurochem.*, **1983**, *41*, 188–194.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate 250 mg rat brain tissue with 6 mL 1 M formic acid containing 10 nmoles IS for 5 min, centrifuge at 4° at 10000 g for 20 min, add the supernatant to an equal volume of diethyl ether, add 5 mL water, shake, centrifuge at 2000 g for 5 min, discard the organic layer. Lyophilize the aqueous layer, dissolve the residue in 400 μL water, filter (0.45 μm). Add 30 μL reagent to the filtrate, mix, centrifuge at 10000 g for 5 min. Dissolve the precipitate in 300 μL water, add 50 mg Dowex 1x8, shake, centrifuge at 10000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Reagent contained 20% KI and 18% iodine in water.)

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil C18
Mobile phase: Buffer (Prepare buffer by dissolving 1.36 g sodium acetate. 3.72 disodium EDTA, 25 mg sodium octyl sulfate, and 1.2 mmoles tetramethylammonium chloride in 900 mL water, adjust pH to 5.0 with 200 mM citric acid, make up to 1 L.)
Column temperature: 37
Flow rate: 0.8
Injection volume: 10
Detector: E, Bioanalytical Systems LC-4B/17, TL-10A platinum electrode +500 mV, Ag/AgCl reference electrode following post-column reaction. The effluent from the column mixed with buffer pumped at 0.5 mL/min and the mixture flowed through an immobilized enzyme reactor to the detector. (Prepare buffer by dissolving 71.6 g $Na_2HPO_4.12H_2O$ and 372 mg disodium EDTA in 900 mL water, adjust pH to 8.5 with NaH_2PO_4, make up to 1 L. Prepare reactor by heating 200-400 mesh porous glass beads (pore size 400 Å, Electronucleonics, Fairfield NJ) in 5% nitric acid, wash with water, dry, add to 10% 3-aminopropyltriethoxysilane in toluene, reflux overnight. Suspend the beads in 2% glutaraldehyde in water at room temperature for 2 h. Dissolve 0.45 mg acetylcholinesterase (type III, EC.3.1.1.7, Sigma) and 16.6 mg choline oxidase (EC.1.1.3.17, Sigma) in 200 μL 50 mM pH 7.0 phosphate buffer containing 10 mM disodium EDTA, add 500 mg activated beads, pack in a 10 × 4 tube.)

CHROMATOGRAM
Retention time: 6
Internal standard: ethylhomocholine (Prepare ethylhomocholine by adding 3-dimethylamino-1-propanol to EtOH, add bromoethane. When reaction is complete add ether, filter off the precipitate and wash it with ether.) (8)
Limit of detection: 100 fmoles

OTHER SUBSTANCES
Extracted: acetylcholine

KEY WORDS
post-column reaction; rat; brain; immobilized enzyme reactor

REFERENCE
Asano,M.; Miyauchi,T.; Kato,T.; Fujimori,K.; Yamamoto,K. Determination of acetylcholine and choline in rat brain tissue by liquid chromatography/electrochemistry using an immobilized enzyme post column reactor, *J.Liq.Chromatogr.*, **1986**, *9*, 199–215.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate rat brain with ten volumes of 1 M formic acid:acetone 15:85 containing IS, centrifuge at 4° at 20000 g. Remove a 500 μL aliquot of the supernatant and add it to 2 mL heptane:chloroform 80:10, vortex. Remove the aqueous layer and add it to 250 μL 3 mg/mL sodium tetraphenylboron in 3-heptanone, vortex. Remove a 200 μL aliquot of the upper organic layer and add it to 50 μL 1 M HCl, vortex. Remove the aqueous layer and evaporate it to dryness under reduced pressure, reconstitute with mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: 100 mM pH 7 KH_2PO_4 containing 10 μg/mL sodium octane sulfate and 600 μg/mL tetramethylammonium chloride
Flow rate: 1
Injection volume: 30
Detector: E, Chromatofield, Pt electrode +0.5 V following post-column reaction. The column effluent flowed through an immobilized enzyme reactor to the detector. (Prepare reactor by heating 200-400 mesh porous glass beads (pore size 350 Å, Sigma) in 5% nitric acid at 100° for 1 min, wash with water, dry, add to 10% 3-aminopropyltriethoxysilane in toluene, heat at 115° for 12 h. Suspend the beads in 2% glutaraldehyde in water at room temperature for 2 h. Dissolve 100 U acetylcholinesterase (type III, electric eel, Sigma) and 100 U choline oxidase (Alcaligenes, Sigma) in 1 mL 50 mM pH 7 phosphate buffer, add 120 mg activated beads, shake periodically, pack in a 20 × 2 tube.)

CHROMATOGRAM
Retention time: 4.5
Internal standard: ethylhomocholine bromide (Prepare ethylhomocholine by adding 3-dimethylamino-1-propanol to EtOH, add bromoethane, when reaction is complete add ether, filter off the precipitate and wash it with ether.) (7)

OTHER SUBSTANCES
Extracted: acetylcholine

KEY WORDS
rat; brain; post-column reaction; immobilized enzyme reactor

REFERENCE
Beley,A.; Zekhnini,A.; Lartillot,S.; Fage,D.; Bralet,J. Improved method for determination of acetylcholine, choline, and other biogenic amines in a single brain tissue sample using high performance liquid chromatography and electrochemical detection, *J.Liq.Chromatogr.*, **1987**, *10*, 2977–2992.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain tissue with 1 mL 50 mM perchloric acid containing 10 nmoles ethylhomocholine for 1 min (Nissei Model US-300T, 300 W, 20 kHz), centrifuge at 20000 g at 4° for 15 min, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 10 × 4 glassy carbon particles IRICA Type CP-2250 (IRICA Instruments) (removes interfering catecholamines but is not essential)
Column: 60 × 4 3 μm Acetylcholine Separation polymeric styrene-based column (Bioanalytical Systems)
Mobile phase: 50 mM pH 8.4 phosphate containing 1 mM disodium EDTA and 0.4 mM sodium 1-octanesulfonate
Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: E, Bioanalytical systems LC-4A, dual platinum electrodes + 0.50 V, Ag/AgCl reference electrode, following a 5 × 4 reactor containing immobilized acetylcholinesterase and choline oxidase

CHROMATOGRAM

Retention time: 2.04
Internal standard: ethylhomocholine (4.07)

OTHER SUBSTANCES

Extracted: acetylcholine

KEY WORDS

rat; brain; post-column reaction; immobilized enzyme reactor

REFERENCE

Ikarashi,Y.; Iwatsuki,H.; Blank,C.L.; Maruyama,Y. Glassy carbon pre-column for direct determination of acetylcholine and choline in biological samples using liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *575*, 29–37.

SAMPLE

Matrix: tissue
Sample preparation: Tissue. Homogenize brain tissue with 10 volumes 400 mM perchloric acid, centrifuge. Remove the supernatant and add it to one tenth the volume of 7.5 M potassium acetate solution, centrifuge. Remove a 100 μL aliquot and take it to dryness in a vacuum centrifuge, dissolve the residue in mobile phase, inject an aliquot. CSF, plasma. Add two volumes of 96% EtOH to CSF or plasma, centrifuge. Remove a 100 μL aliquot and take it to dryness in a vacuum centrifuge, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 60 × 4.6 5 μm Bakerbond Sulfopropyl
Mobile phase: 100 mM pH 7.5 sodium phosphate with 5 mM tetramethylammonium chloride
Flow rate: 1.2
Detector: E, Biometra EP 20, platinum electrode + 0.5 V following an immobilized enzyme reactor containing choline oxidase (EC 1.1.3.17) and acetylcholine esterase (EC 3.1.1.7) to convert acetylcholine and choline to hydrogen peroxide which was then detected

CHROMATOGRAM

Retention time: 2.1
Limit of detection: 0.1 pmol

OTHER SUBSTANCES

Extracted: acetylcholine

KEY WORDS

rat; brain; post-column reaction; immobilized enzyme reactor

REFERENCE

Klein,J.; Gonzalez,R.; Köppen,A.; Löffelholz,K. Free choline and choline metabolites in rat brain and body fluids: sensitive determination and implications for choline supply to the brain, *Neurochem.Int.*, **1993**, *22*, 293–300.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Nissei US-300T ultrasonic cell disrupter at 300 W and 20 kHz for 1 min) rat brain striatal tissue with 1 mL 1 μM ethylhomocholine in 50 mM perchloric acid, centrifuge at 4° at 20000 g for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 74-149 μm plastic formed carbon (details in paper)

Column: 60 × 4 3 μm Acetylcholine Separation polymeric styrene column (Bioanalytical Systems)

Mobile phase: 50 mM pH 8.40 Phosphate buffer containing 1 mM disodium EDTA and 0.40 mM sodium 1-octanesulfonate

Column temperature: 35 ± 1

Flow rate: 0.7

Injection volume: 10

Detector: E, Bioanalytical Systems LC-4A, dual Pt working electrode +500 mV, Ag/AgCl reference electrode following post-column reaction. The effluent from the column flowed through a 5 × 4 immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (Bioanalytical Systems) to the detector.

CHROMATOGRAM

Retention time: 3.5

Internal standard: ethylhomocholine (6.5)

OTHER SUBSTANCES

Extracted: acetylcholine

Noninterfering: 3,4-dihydroxybenzylamine, dopamine, norepinephrine

KEY WORDS

rat; brain; guard-column removes interferences from catecholamines; post-column reaction; immobilized enzyme reactor

REFERENCE

Ikarashi,Y.; Blank,C.L.; Suda,Y.; Kawakubo,T.; Maruyama,Y. Application of a novel, plastic formed carbon as a precolumn packing material for the liquid chromatographic determination of acetylcholine and choline in biological samples, *J.Chromatogr.A*, **1995**, *718*, 267–272.

Chymopapain

Molecular formula: indefinite
CAS Registry No.: 9001-09-6
Merck Index: 2319

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 7.5 TSK SP 5PW (Toyo Soda)
Mobile phase: Gradient. 20 mM pH 5.0 sodium acetate buffer:100 mM pH 5.0 sodium acetate buffer 70:30 to 0:100 over 75 min
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 19, 44 (most active fractions)

KEY WORDS
commercial chymopapain is a complex mixture

REFERENCE
Calam,D.H.; Davidson,J.; Harris,R. High-performance liquid chromatographic investigations on some enzymes of papaya latex, *J.Chromatogr.*, **1985**, *326*, 103–111.

Chymotrypsin

Molecular formula: indefinite
CAS Registry No.: 9004-07-3
Merck Index: 2320

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 2.6 ODS-HC/Sil-X-1 (Perkin-Elmer)
Mobile phase: Gradient. MeCN containing 0.07% trifluoroacetic acid:0.1% trifluoroacetic acid in water from 0:100 to 60:40 over 30 min
Flow rate: 2
Detector: UV 206

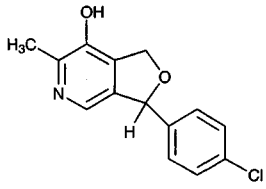
CHROMATOGRAM

Retention time: 25

REFERENCE

Titani,K.; Sasagawa,T.; Resing,K.; Walsh,K.A. A simple and rapid purification of commercial trypsin and chymotrypsin by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1982**, *123*, 408–412.

Cicletanine



Molecular formula: $C_{14}H_{12}ClNO_2$
Molecular weight: 261.71
CAS Registry No.: 89943-82-8, 82747-56-6 (HCl)
Merck Index: 2323
Lednicer No.: 5 143

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 200 μL 500 ng/mL IS in 250 mM pH 2.5 citrate buffer, inject a 50 μL aliquot onto column A with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B, after 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B, re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 50 × 2 37-50 μm Bondapak Cx/Corasil (ion-exchange); B 23 × 3.9 37-50 μm Bondapak C18/Corasil + 100 × 8 4 μm Nova-Pak C18 Radial Pak
Mobile phase: A water; B MeCN:100 mM pH 2.5 KH_2PO_4 25:75
Flow rate: A 1; B 2
Injection volume: 50
Detector: F ex 290 em >370 (filter)

CHROMATOGRAM

Retention time: 5.3
Internal standard: 3-(4-chlorophenyl)-1H-3,6-dimethylfuro[3,4-c]pyridin-7-ol (7.3)
Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Antoniewicz,S.M.; Cook,J.A.; Brown,R.R. Determination of cicletanine in human plasma by high-performance liquid chromatography using automated column switching, *J.Chromatogr.*, **1992**, *573*, 93–98.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 μg/mL IS in MeOH:water 10:90 + 7 mL diethyl ether, shake mechanically for 10 min, centrifuge at 1000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, extract the aqueous layer with another 5 mL diethyl ether, remove 5 mL of the organic layer and add it to the residue from the first organic layer. Evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residues in 200 μL MeCN:water 10:90, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 5 × 4 4 μm Nova-Pak C18
Column: 100 × 8 4 μm Nova-Pak C18 Radial Pak
Mobile phase: MeCN:water 22:78 containing 0.02% isopropylamine and 0.085% phosphoric acid, pH 2.4 (Wash column with MeCN for 3 min at the end of each injection.)
Flow rate: 2.5
Injection volume: 100
Detector: UV 220

CHROMATOGRAM

Retention time: 10
Internal standard: (±)-2-methyl-3-hydroxy-4H,5H-5-methyl-(4'-chlorophenyl)isofuropyridine hydrochloride (17.5)
Limit of detection: 10 ng/mL

KEY WORDS

comparison with capillary electrophoresis; plasma; pharmacokinetics

REFERENCE

Pruñonosa,J.; Obach,R.; Diez-Cascón,A.; Gouesclou,L. Comparison of high-performance liquid chromatography and high-performance capillary electrophoresis for the determination of cicletanine in plasma, *J.Chromatogr.*, **1992**, *581*, 219–226.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Retention time: 4.70
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; na-

proxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.808

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: urine

Sample preparation: 200 μL Urine + 20 μL 10 μg/mL methylcicletanine, adjust pH to 5.2 with 50 μL 1 M acetate buffer, add 2 mL hexane:ether 80:20, vortex, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot. (Hydrolyze sulfate conjugates by heating 200 μL urine with 400 μL 1 M HCl at 37° for 12 h. Hydrolyze glucuronide conjugate with β-glucuronidase (E.C.3.21.31, Helix pomatia type H-1, Sigma) at 37° at pH 5.2 for 12 h.)

HPLC VARIABLES

Column: 250 × 4 5 μm ChiraDex (Merck)

Mobile phase: MeOH:water 30:70 containing 0.1% triethylammonium chloride, pH adjusted to 4.0

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Internal standard: methylcicletanine

Limit of detection: 1 μg/mL

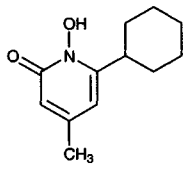
KEY WORDS

chiral; rat

REFERENCE

Vistelle,R.; Lamiable,D.; Morin,E.; Trenque,T.; Kaltenbach,M. Urinary excretion of cicletanine in the rat. Stereochemical aspects, *Drug Metab.Dispos.*, **1995**, *23*, 988–992.

Ciclopirox



Molecular formula: $C_{12}H_{17}NO_2$
Molecular weight: 207.27
CAS Registry No.: 29342-05-0, 41621-49-2 (olamine)
Merck Index: 2325

SAMPLE

Matrix: blood
Sample preparation: Condition an Adsorbex CN SPE cartridge (E. Merck) with 1 mL MeCN. 1 mL Plasma + 1 mL 1/15 M pH 5.0 phosphate buffer (KH_2PO_4 and Na_2HPO_4) + 10 µL 40 IU/mL β-glucuronidase, incubate at 37° for 24 h, add 500 µL 200 mM NaOH, add 200 µL dimethyl sulfate (CAUTION! Highly Toxic!), heat at 37° for 20 min, add 200 µL triethylamine, add 10 mL n-hexane, extract. Add 8 mL of the organic phase to the SPE cartridge, wash with 1 mL toluene, aspirate to dryness under reduced pressure for 3 min, elute with 350 µL mobile phase, evaporate eluate at 40° under nitrogen, dissolve residue in 80 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 5 µm LiChrospher 100 RP18 in a LiChroCART 4-4
Column: 125 × 4 5 µm LiChrospher 100 RP18
Mobile phase: MeCN:water 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 304

CHROMATOGRAM

Retention time: 7.6
Limit of detection: 15 ng/mL

KEY WORDS

plasma; SPE; rabbit; derivatization; pharmacokinetics

REFERENCE

Coppi,G.; Silingardi,S. HPLC method for pharmacokinetic studies on ciclopirox olamine in rabbits after intravenous and intravaginal administrations, *Farmaco*, **1992**, *47*, 779–786.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve 1 g cream, lotion, or shampoo in 100 mL MeOH:water 70:30 with sonication, centrifuge, filter (0.45 µ), inject a 10 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm Purospher RP-18 endcapped (Merck)
Mobile phase: MeCN:water containing 20 mM phosphoric acid and 500 µM disodium EDTA 68:32
Flow rate: 1
Injection volume: 10
Detector: UV 305

CHROMATOGRAM

Retention time: 4.25

OTHER SUBSTANCES

Simultaneous: metabolites, octopirox

KEY WORDS
cream; lotion; shampoo

REFERENCE
Gagliardi,L.; Multari,G.; Cavazzutti,G.; De Orsi,D.; Tonelli,D. HPLC determination of ciclopirox, octopirox, and pyrithiones in pharmaceuticals and antidandruff preparations, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2365–2373.

SAMPLE
Matrix: formulations
Sample preparation: Foam. Weigh out an amount equivalent to about 10 mg ciclopirox, add 2 mL 1 M NaOH, add 30 μL methyl iodide, vortex, keep in an ice bath for 10 min, add 30 μL 25% ammonium hydroxide, dilute to 50 mL with MeCN:water 1:1, inject a 0.2 μL aliquot. Powder. Weigh out an amount equivalent to about 2 mg ciclopirox, add 2 mL MeCN:water 1:1, add 1 mL 1 M NaOH, add 20 μL methyl iodide, vortex, keep in an ice bath for 10 min, add 20 μL 25% ammonium hydroxide, dilute to 50 mL with MeCN:water 1:1, inject a 0.2 μL aliquot.

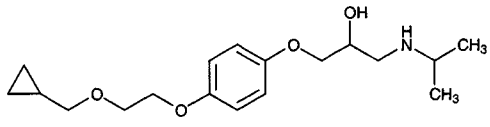
HPLC VARIABLES
Column: 150 × 0.33 5 μm 300 Å DeltaPak RP-18 fused silica capillary (Fusica, LC Packings)
Mobile phase: MeCN:water 50:50
Column temperature: 20
Flow rate: 0.01
Injection volume: 0.2
Detector: UV 300

CHROMATOGRAM
Retention time: 2
Limit of detection: 10 ng

KEY WORDS
foam; powder; capillary HPLC; derivatization

REFERENCE
Belliardo,F.; Bertolino,A.; Brandolo,G.; Lucarelli,C. Micro-liquid chromatography method for the determination of ciclopiroxolamine after pre-column derivatization in topical formulations, *J.Chromatogr.*, **1991**, *553*, 41–45.

Cicloprolol



Molecular formula: $C_{18}H_{29}NO_4$
Molecular weight: 323.44
CAS Registry No.: 94651-09-9, 63659-12-1 (without (±)-definition), 63686-79-3 (HCl)
Lednicer No.: 4 25

SAMPLE
Matrix: solutions

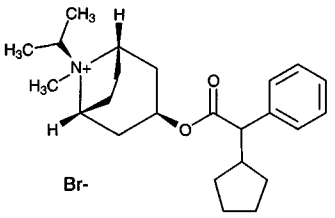
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 10.28

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Ciclotropium bromide



Molecular formula: $C_{24}H_{36}BrNO_2$
Molecular weight: 450.46
CAS Registry No.: 85166-20-7

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 4 mL Plasma + 5.5 mL chloroform + 500 µL reagent A + 250 µL 1 M HCl, shake vigorously for 5 min, centrifuge at 10° at 2500 g for 30 min. Remove a 4 mL aliquot of the organic layer and add it to 2.4 mL 100 mM HCl, shake for 15 min, centrifuge at 20° at 2500 g for 10 min. Remove a 2 mL aliquot of the aqueous layer and add it to 1 mL 1 M NaOH, heat at 140-5° for 90 min (to hydrolyse ciclotropium), cool to room temperature, add 500 µL reagent A, adjust pH to 8.5-9.5 with 5 M HCl, add 2.6 mL chloroform, shake for 15 min, centrifuge at 20° at 2500 g for 10 min. Remove a 2 mL aliquot of the chloroform layer and add it to 1.4 mL 100 mM HCl, shake for 15 min, centrifuge at 20° at 2500 g for 10 min. Remove a 1 mL aliquot of the aqueous layer and add it to 1 mL MeOH, evaporate to dryness under reduced pressure, add three 3 mL portions of MeOH and evaporate to dryness each time, take up the residue in 200 µL 10 mg/mL flunoxaprofen chloride in MeCN (freshly prepared), heat at 110° for 15 min, evaporate to dryness under reduced pressure, add 1 mL ethyl acetate, add 1.3 mL 10 mM HCl, shake vigorously for 15 min, centrifuge at 20° at 2500 g for 10 min, discard the organic layer, wash the aqueous layer twice more with ethyl acetate, evaporate the aqueous layer to dryness under reduced pressure, reconstitute with 100 µL MeCN:MeOH: water 1:1:1, inject a 20 µL aliquot. Urine. 1 mL Urine + 100 µL 1 µg/mL IS in water + 2 mL 0.5 M NaOH, heat at 140-5° for 90 min (to hydrolyse ciclotropium), cool to room temperature, add 500 µL reagent B, adjust pH to 8.5-9.5 with 5 M HCl, add 2.6 mL chloroform, shake for 15 min, centrifuge at 20° at 2500 g for 10 min. Remove a 2 mL aliquot of the chloroform layer and add it to 1.4 mL 100 mM HCl, shake for 15 min, centrifuge at 20° at 2500 g for 10 min. Remove a 1 mL aliquot of the aqueous layer and add it to 1 mL MeOH, evaporate to dryness under reduced pressure, add three 3 mL portions of MeOH and evaporate to dryness each time, take up the residue in 200 µL 10 mg/mL flunoxaprofen chloride in MeCN (freshly prepared), heat at 110° for 15 min, evaporate to dryness under reduced pressure, add 1 mL ethyl acetate, add 1.3 mL 10 mM HCl, shake vigorously for 15 min, centrifuge at 20° at 2500 g for 10 min, discard the organic layer, wash the aqueous layer twice more with ethyl acetate, evaporate the aqueous layer to dryness under reduced pressure, reconstitute with 500 µL MeCN:MeOH: water 1:1:1, inject a 20 µL aliquot. (Prepare reagent A by mixing 100 mg dipicrylamine, 600 mg anhydrous sodium carbonate, and 10 mL water. Prepare reagent B by mixing 35 mg dipicrylamine and 10 mL 100 mM NaOH. Prepare dipicrylamine as follows (Caution! Dipicrylamine is potentially explosive and highly toxic, store moistened with 50% water!). Add 50 g 2,4-dinitrodiphenylamine to 420 g nitric acid (36° Bé. 52%, d = 1.33) heated to 62° over 2 h, heat at 62-90° for another 3 h, cool, filter, wash the product until it is free of acid, dry to obtain 2,2',4,4'-tetranitrodiphenylamine as a yellow solid (mp 187.4°). Add 50 g tetranitrodiphenylamine over 1 h to 500 g of a mixture of equal parts 92% sulfuric acid and 93% nitric acid at room temperature, after 4.5 h add to a large volume of ice water, filter, recrystallize the product from acetone to obtain dipicrylamine (2,2',4,4',6,6'-hexanitrodiphenylamine) as yellow crystals (mp 242.9°) (J.Am.Chem.Soc. 1919, 41, 1013). Prepare flunoxaprofen chloride as follows. Dissolve 1 mmole flunoxaprofen in 25 mL toluene, add a trace of DMF (J.Chromatogr. 1990, 528, 55), add 2.5 mL thionyl chloride, reflux for 30 min, remove solvent by evaporation, dry the residue under vacuum over KOH, recrystallize from dichloromethane (mp 73°) (J.Chromatogr. 1988, 427, 131).)

HPLC VARIABLES

Column: 150 × 3.9 5 µm Suplex pkb-100 (Supelco)

Mobile phase: MeCN:water 55:45 containing 1 mL/L 50% phosphoric acid and 0.6 g/L dodecyl sulfate.
Flow rate: 1
Injection volume: 20
Detector: F ex 310 em 365

CHROMATOGRAM

Retention time: 8.0
Internal standard: N-butyltropinium (11.1)
Limit of detection: 0.5 ng/mL (plasma), 10 ng/mL (urine)

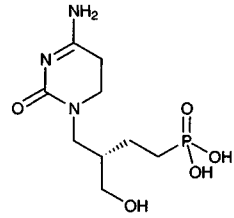
KEY WORDS

derivatization; plasma; pharmacokinetics

REFERENCE

Liebmann,B.; Henke,D.; Spahn-Langguth,H.; Mutschler,E. Determination of the quaternary compound ciclotropium in human biological material after hydrolysis and derivatization with the fluorophor flunoxaprofen chloride, *J.Chromatogr.*, **1991**, *572*, 181–193.

Cidofovir



Molecular formula: $C_8H_{14}N_3O_6P$
Molecular weight: 279.19
CAS Registry No.: 113852-37-2
Merck Index: 2329

SAMPLE

Matrix: blood
Sample preparation: Briefly vortex 100 µL plasma with 300 µL 500 ng/mL IS in MeCN: water:acetic acid 80:19:1, centrifuge at 15000 g for 5 min. Remove the supernatant and add it to 100 µL 1.25 mM phenacyl bromide in MeCN, heat at 80° for 45 min, evaporate to dryness under reduced pressure at room temperature, reconstitute with 60 µL water, vortex briefly, centrifuge at 15000 g for 5 min, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.6 5 µm Prodigy ODS-2 (Phenomenex)
Mobile phase: MeCN:water 30:70 containing 12 mM phosphoric acid and 6 mM dodecyltriethylammonium phosphate (Q12) (Bodman) (final pH 3.0-3.1)
Column temperature: 45
Flow rate: 3
Injection volume: 20
Detector: F ex 305 em 370

CHROMATOGRAM

Retention time: 3.1
Internal standard: cytidine-5'-monophosphate (5.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: lamivudine, zalcitabine

KEY WORDS

derivatization; monkey; plasma

REFERENCE

Eisenberg,E.J.; Cundy,K.C. High-performance liquid chromatographic determination of cytosine-containing compounds by precolumn fluorescence derivatization with phenacyl bromide: application to antiviral nucleosides and nucleotides, *J.Chromatogr.B*, **1996**, *679*, 119–127.

SAMPLE

Matrix: dialysate

HPLC VARIABLES

Column: C18
Mobile phase: MeCN:MeOH:2.5 mM pH 3.0 ammonium dihydrogen phosphate buffer 17.5: 17.5:65
Detector: UV 278

CHROMATOGRAM

Retention time: 2.5
Internal standard: tryptophan (4.2)
Limit of detection: 10 ng/mL

KEY WORDS

pharmacokinetics; rabbit; microdialysis

REFERENCE

Duggirala,S.M.; Mitra,A.K. Intravitreal pharmacokinetics of anti CMV agents ganciclovir and cidofovir -a comparison (Abstract 1119), *Pharm.Res.*, **1997**, *14*, S39–S39.

SAMPLE

Matrix: formulations
Sample preparation: If necessary, dilute injection 1:50 with water, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 (Alltech Associates S/N 94091346)
Column: 150 × 4.6 5 μm Hypersil octadecylsilane C18
Mobile phase: 3.5 mM Na_2HPO_4 containing 5 mM tetrabutylammonium dihydrogen phosphate, adjusted to pH 6.0 with concentrated phosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: degradation products

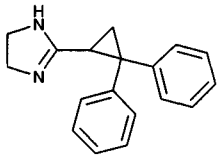
KEY WORDS

5 % dextrose; 0.9 % sodium chloride; injections; stability-indicating

REFERENCE

Yuan,L.-C.; Samuels,G.J.; Visor,G.C. Stability of cidofovir in 0.9% sodium chloride injection and in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1939–1943.

Cifenline



Molecular formula: $C_{18}H_{18}N_2$
Molecular weight: 262.35
CAS Registry No.: 53267-01-9, 100678-32-8 (succinate)
Merck Index: 2330
Lednicer No.: 4 87

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 250 μL triethylamine + 100 μL 1 M NaOH + 100 μL 15 μg/mL p-chlorodisopyramide in 10 mM HCl + 1.75 mL dichloromethane, rotate slowly for 20 min, centrifuge at 3600 g for 20 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 10 mM HCl, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 5 μm Nucleosil CN
Mobile phase: MeCN:buffer 35:65 (Buffer was 25 mL PIC B-8 (1-octanesulfonic acid), 2.5 mL PIC D-4 (dibutylamine phosphate), 1 mL butylamine, and 971.5 mL water.)
Flow rate: 1.1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 11.5
Internal standard: p-chlorodisopyramide (18.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: aspirin, betaxolol, diazepam, digitoxin, dihydralazine, enalapril, furosemide, glibenclamide, heparin, hydrochlorothiazide, isosorbide mononitrate, metildigoxin, metoprolol, nifedipine, nitrendipine, phenprocoumon, ranitidine, spironolactone, triamterene, verapamil, xipamide

KEY WORDS

serum

REFERENCE

Kühlkamp,V.; Schmid,F.; Ress,K.M.; Krämer,B.K.; Mayer,F.; Liebich,H.M.; Risler,T.; Seipel,L. Quantification of cibenzoline and its imidazole metabolite by high-performance liquid chromatography in human serum, *J.Chromatogr.*, **1990**, *528*, 267–273.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 5.69
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma or urine + 50 μL 2 μg/mL IS in MeCN + 2 mL buffer, vortex, add 2.5 mL benzene (CAUTION! Benzene is a carcinogen!), shake on a reciprocating shaker at 80-100 strokes/min for 10 min, centrifuge at 1460 g at 10° for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 65°, reconstitute the residue in 400 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7-8 μm Zorbax SCX

Mobile phase: MeCN:buffer 80:20 (Prepare buffer from 6 mL 1 M pH 6.0 phosphate buffer and 394 mL water. Prepare 1 M pH 6.0 phosphate buffer from 430 mL 1 M orthophosphoric acid + 570 mL 1 M KH_2PO_4, adjust pH to 6.0.) (Flush column with MeCN:water 80:20 at the end of each day.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 214

CHROMATOGRAM

Retention time: 5.5

Internal standard: 2-[2,2-bis(4-methylphenyl)-1-cyclopropyl]-4,5-dihydro-1H-imidazole (4.5)

Limit of detection: 10 ng/mL (plasma)

Limit of quantitation: 50 ng/mL (urine)

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Hackman,M.R.; Lee,T.L.; Brooks,M.A. Determination of cibenzoline in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *273*, 347–356.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.143

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% NAPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (NAPIC is (-)-(S)-naproxen isocyanate; synthesis is as follows (protect from light). Dissolve 1 g (+)-(S)-naproxen in 30 mL acetone, cool to 0°, add a solution of 700 μL triethylamine in 2 mL acetone dropwise, add a solution of 450 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 310 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain NAPIC as an oil that crystallized in the desiccator (mp 48°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Nucleosil phenyl
Mobile phase: MeOH:water:diethylamine 63:37:0.05
Flow rate: 1
Injection volume: 20
Detector: F ex 276 em 356

CHROMATOGRAM
Retention time: 15.1 (S), 20.6 (R)

KEY WORDS
derivatization; chiral

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution

of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES

Column: 250 × 4.6 7 μm Nucleosil phenyl (A) or 200 × 4.6 5 μm Nucleosil cyano (B)
Mobile phase: MeOH:80 mM NaCl in water 68:32 (A) or n-hexane:isopropanol 88:12 (B)
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM

Retention time: 18.4 (R (A)), 22.4 (S (A)), 15.9 (R (B)), 18.6 (S (B))

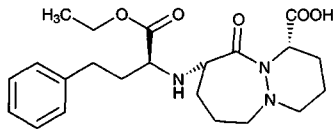
KEY WORDS

derivatization; chiral

REFERENCE

Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

Cilazapril



Molecular formula: $C_{22}H_{31}N_3O_5.H_2O$
Molecular weight: 435.52
CAS Registry No.: 92077-78-6, 88768-40-5 (anhydrous)
Merck Index: 2332
Lednicer No.: 4 170

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.367

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations
Sample preparation: Grind tablets to a fine powder. Weigh out amount equivalent to 25 mg cilazapril, extract with MeOH, filter. Mix 400-2000 μL filtrate with 500 μL 4 mg/mL IS in MeOH, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 20

Detector: UV 211

CHROMATOGRAM
Retention time: 17.61
Internal standard: enalapril (8.58)
Limit of detection: 10 μg/mL
Limit of quantitation: 40 μg/mL

OTHER SUBSTANCES
Simultaneous: benazepril

KEY WORDS
tablets

REFERENCE
Gumieniczek,A.; Przyborowski,L. Determination of benazepril and cilazapril in pharmaceuticals by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2135–2142.

SAMPLE
Matrix: solutions

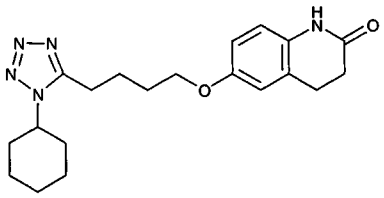
HPLC VARIABLES
Column: 250 × 4.6 Spherisorb 5 ODS-2
Mobile phase: n-Propanol:buffer 20:80 (Buffer was pH 3.0 phosphate buffer containing 0.4% triethylamine.)
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: quinapril, captopril, enalapril, benzepril, ramipril

REFERENCE
Barbato,F.; Morrica,P.; Quaglia,F. Analysis of ACE inhibitor drugs by high performance liquid chromatography, *Farmaco*, **1994**, *49*, 457–460.

Cilostazol



Molecular formula: $C_{20}H_{27}N_5O_2$
Molecular weight: 369.47
CAS Registry No.: 73963-72-1
Merck Index: 2335

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 60 μg/mL IS in MeOH + 4 mL MeCN, vortex, centrifuge at 1700 g for 10 min. Remove the supernatant and evaporate the MeCN under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL chloroform, shake, centrifuge at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL diethyl ether, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 42:58
Flow rate: 1.7
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5
Internal standard: 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)propoxy]-3,4-dihydro-1-ethyl-2(1H)-quinolinone (OPC-13012) (12)
Limit of quantitation: 25 ng/mL

KEY WORDS

plasma; pharmacokinetics; human; dog (Arzneimittelforschung 1985; 35; 1124)

REFERENCE

Akiyama,H.; Kudo,S.; Odomi,M.; Shimizu,T. High-performance liquid chromatographic procedure for the determination of a new antithrombotic and vasodilating agent, cilostazol, in human plasma, *J.Chromatogr.*, **1985**, *338*, 456–459.

SAMPLE

Matrix: blood
Sample preparation: Rat. 1 mL Plasma + 10 μL 20 μg/mL IS in MeOH + 3 mL EtOH, vortex, centrifuge at 1700 g for 10 min. Remove the supernatant and evaporate the EtOH under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL ethyl ether, shake, centrifuge at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot. Rabbit. 500 μL Plasma + 5 μL 20 μg/mL IS in MeOH + 500 μL water + 3 mL EtOH, vortex, centrifuge at 1700 g for 10 min. Remove the supernatant and evaporate the EtOH under a stream of air, reconstitute the residue in 1 mL 200 mM NaOH, add 5 mL ethyl ether, shake, centrifuge at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 42:58

Flow rate: 1.7
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Internal standard: 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)propoxy]-3,4-dihydro-1-ethyl-2(1H)-quinolinone (OPC-13012)
Limit of quantitation: 25 ng/mL

KEY WORDS

rat; rabbit; plasma; pharmacokinetics

REFERENCE

Akiyama,H.; Kudo,S.; Shimizu,T. The absorption, distribution and excretion of a new antithrombotic and vasodilating agent, cilostazol, in rat, rabbit, dog and man, *Arzneimittelforschung*, **1985**, *35*, 1124–1132.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 50 mg in 50 mL MeOH:water 50:50. Remove a 1 mL aliquot and add it to 1 mL 0.05% benzophenone in EtOH, make up to 10 mL with MeOH:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM potassium nitrate 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Internal standard: benzophenone

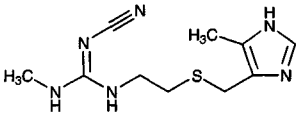
OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Shimizu,T.; Osumi,T.; Niimi,K.; Nakagawa,K. Physico-chemical properties and stability of cilostazol, *Arzneimittelforschung*, **1985**, *35*, 1117–1123.

Cimetidine



Molecular formula: $C_{10}H_{16}N_6S$
Molecular weight: 252.34
CAS Registry No.: 51481-61-9, 70059-30-2 (HCl)
Merck Index: 2337
Lednicer No.: 2 253; 4 89, 95, 112

SAMPLE

Matrix: blood, CSF, tissue
Sample preparation: Plasma. 25 μL Plasma + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 1650 g for 10 min. Evaporate 4 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase. Inject a 25 μL aliquot. Tissue. Homogenize brain tissue with 1 mL saline on ice for 1 min. Add 100 μL 1 M NaOH, extract with 5 mL dichloromethane. Evaporate a 3 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase, centrifuge at 10000 g. Inject a 25 μL aliquot. CSF. Inject a 25 μL aliquot of the CSF directly.

HPLC VARIABLES

Column: 250 × 4 Senshu gel 5C18H (Senshu, Japan)
Mobile phase: MeCN:5 mM NaH_2PO_4 containing 5 mM tetramethylammonium chloride 5:95
Column temperature: 40
Flow rate: 2
Injection volume: 25
Detector: UV 320

CHROMATOGRAM

Internal standard: cimetidine

OTHER SUBSTANCES

Extracted: ranitidine

KEY WORDS

plasma; brain; rat; cimetidine is IS

REFERENCE

Nakada,Y.; Yamamoto,K.; Kawakami,J.; Sawada,Y.; Iga,T. Effect of renal failure on neurotoxicity of ranitidine in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 323–325.

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 75 μM IS + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Evaporate 4 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 400 μL mobile phase, inject a 20 μL aliquot. Tissue. Homogenize 500 mg liver with saline on ice for 1 min. Add 100 μL 75 μM IS, 100 μL 0.5 M NaOH, and 5 mL dichloromethane, shake for 10 min, centrifuge at 3000 rpm for 10 min. Evaporate 3 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 400 μL mobile phase, pass through a Ministar-RC 15 cartridge (Sartorius, Germany), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Senshu Pak ODS-1031 (Senshu Sciences, Japan)
Column: 250 × 4.6 Senshu Pak ODS -1251 (Senshu Sciences, Japan)

Mobile phase: MeCN:water 5:95 containing 5 mM NaH_2PO_4 and 5 mM tetramethylammonium chloride
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Internal standard: nizatidine
Limit of detection: 50-100 μg/mL (sic)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.602

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 2.71

OTHER SUBSTANCES
Simultaneous: cisplatin (UV 198), dacarbazine (UV 300), granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:water containing 0.03% phosphoric acid 20:80
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 6.7

OTHER SUBSTANCES
Simultaneous: clomipramine, halofantrine, haloperidol, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions

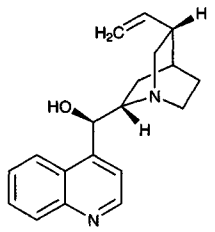
HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: atenolol, hydrochlorothiazide, ranitidine

KEY WORDS
tritium labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

Cinchonidine



Molecular formula: $C_{19}H_{22}N_2O$
Molecular weight: 294.40
CAS Registry No.: 485-71-2
Merck Index: 2345

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, prom-

azine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

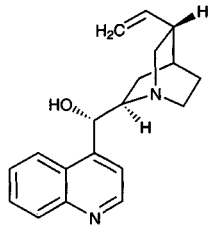
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazo-

cine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cinchonine



Molecular formula: $C_{19}H_{22}N_2O$
Molecular weight: 294.40
CAS Registry No.: 118-10-5
Merck Index: 2346

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM

Retention time: 10.198

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

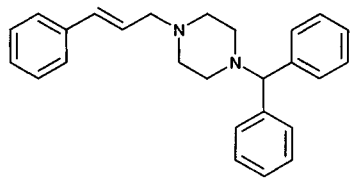
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cinnarizine



Molecular formula: $C_{26}H_{28}N_2$
Molecular weight: 368.52
CAS Registry No.: 298-57-7
Merck Index: 2365
Lednicer No.: 1 58

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 19.258

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 177.82

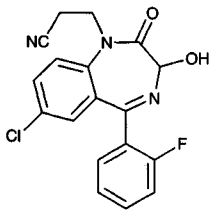
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Cinolazepam



Molecular formula: $C_{18}H_{13}ClFN_3O_2$
Molecular weight: 357.77
CAS Registry No.: 75696-02-5
Merck Index: 2368

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: not given
Mobile phase: MeCN:buffer 44:56 (Buffer was MeCN:50 mM pH 2.5 sodium heptanesulfonate 5:95.)
Detector: UV 230

KEY WORDS
tablets

REFERENCE
Oelschläger,H.; Volke,J.; Belal,F. Analysis of drugs by polarography, XXXV: The polarographic behaviour of cinolazepam [1-(2-cyanoethyl)-7-chloro-3-hydroxy-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one] and assay of its tablets, *Arch.Pharm.(Weinheim)*, **1992**, *325*, 65–68.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, inject an alaiquot.

HPLC VARIABLES
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeCN:10 mM orthophosphoric acid 30:70
Detector: UV 230

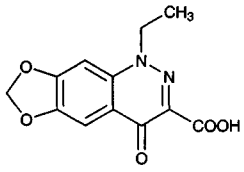
CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Extracted: oxazepam, glucuronides

KEY WORDS
human; rabbit

REFERENCE
Mascher,H.; Nitsche,V.; Schütz,H. Separation, isolation and identification of optical isomers of 1, 4-benzodiazepine glucuronides from biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *306*, 231–239.

Cinoxacin



Molecular formula: $C_{12}H_{10}N_2O_5$
Molecular weight: 262.22
CAS Registry No.: 28657-80-9
Merck Index: 2369
Lednicer No.: 2 388

SAMPLE

Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 268

CHROMATOGRAM

Retention time: 9.88
Internal standard: rosoxacin (5.79)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with deionized water. Adjust to pH 2.5-3 with HCl, extract with 25 mL chloroform. Separate the organic layer, dry the organic phase with sodium sulfate, evaporate it to dryness. Dissolve the residue in 3 mL MeCN, dilute to 10 mL with water, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:0.4 mM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: F ex 270 em 440

CHROMATOGRAM

Retention time: 1.64
Limit of detection: 2.05 ng/mL

OTHER SUBSTANCES

Extracted: oxolinic acid, pipemidic acid

REFERENCE

Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

SAMPLE

Matrix: urine

Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18

Mobile phase: MeCN:400 μM oxalic acid in water 28:72

Flow rate: 2.0

Injection volume: 20

Detector: UV 265

CHROMATOGRAM

Retention time: 1.64

Limit of detection: 700 ng/mL

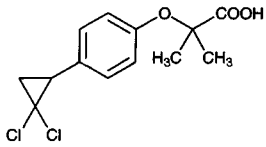
OTHER SUBSTANCES

Simultaneous: nalidixic acid, oxolinic acid, pipemidic acid, piromidic acid

REFERENCE

Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Ciprofibrate



Molecular formula: $C_{13}H_{14}Cl_2O_3$
Molecular weight: 289.16
CAS Registry No.: 52214-84-3
Merck Index: 2373
Lednicer No.: 3 44

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 100 µg/mL IS in ethyl acetate + 2 mL 1 M HCl + 200 µL 60% perchloric acid, extract twice with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen using a warm water bath, reconstitute the residue in 2 mL MeCN, add 2 mL hexane, shake vigorously. Remove the MeCN layer and evaporate it to dryness under a stream of nitrogen using a warm water bath, reconstitute the residue in 500 µL MeCN:THF 10:1, add 500 µL 100 mM pH 4 K_2HPO_4, shake vigorously, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 25 × 3.9 37-50 µm Bondapak phenyl/Corasil
Column: 300 × 3.9 10 µm C2-phenyl (Waters)
Mobile phase: MeCN:THF:100 mM pH 4 K_2HPO_4 96:10:104
Flow rate: 2
Injection volume: 100
Detector: UV 232

CHROMATOGRAM

Retention time: 4
Internal standard: 2-[4-(2,2-dichloro-3-phenylcyclopropyl)phenoxy]-2-methylpropanoic acid (6)
Limit of quantitation: 690 ng/mL

KEY WORDS

plasma; human; rat

REFERENCE

Park,G.B.; Biddlecome,C.E.; Koblantz,C.; Edelson,J. Determination of ciprofibrate in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *227*, 534–539.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 21.22

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 800 μL chloroform:MeOH 2:1, agitate vigorously for 1 min, centrifuge, inject a 5 μL aliquot of the upper water/MeOH layer.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 55:45 containing 9 mM KH_2PO_4, pH 5.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Extracted: ATP, metabolites

KEY WORDS
rat; liver

REFERENCE
Bronfman,M.; Amigo,L.; Morales,M.N. Activation of hypolipidaemic drugs to acyl-coenzyme A thioesters, *Biochem.J.*, **1986**, *239*, 781–784.

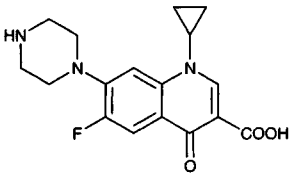
SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 12 with NaOH, heat at 40° for 30 min, acidify to pH 3.0 extract twice with 5 mL n-hexane. Evaporate the combined extracts, reconstitute in 1 mL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:5 mM pH 3.3 PIC B7 (heptanesulfonic acid) 50:50
Flow rate: 1
Detector: UV 230

REFERENCE

Oelschläger,H.; Rothley,D.; Hellwich,K.-H.; Schmidt,W. Zur Pharmakokinetik von Lipidsenkern, 6. Mitt. Ist 2-(4-(2,2-Dichlorocyclopropyl)-phenoxy)-propan ein Metabolit des lipidsenkers Ciprofibrat? [The pharmacokinetics of hypolipidemic agents, VI: Is 2-(4-(2,2-dichlorocyclopropyl)-phenoxy)-propane a metabolite of the hypolipidemic agent ciprofibrate?], *Arch.Pharm.(Weinheim)*, **1989**, *322*, 629–632.

Ciprofloxacin



Molecular formula: $C_{17}H_{18}FN_3O_3$
Molecular weight: 331.35
CAS Registry No.: 85721-33-1, 86393-32-0 (hydrochloride monohydrate)
Merck Index: 2374
Lednicer No.: 4 141

SAMPLE

Matrix: aqueous humor
Sample preparation: Add 350 μL distilled water and 1 μg/mL pipemidic acid solution to 50 μL aqueous humor, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak with Novapak C18 insert
Column: 100 × 8.0 4 μm Novapak C18
Mobile phase: MeCN:MeOH:400 mM citric acid 7.14:21.43:71.43
Flow rate: 1
Injection volume: 20
Detector: F ex 278 em 450

CHROMATOGRAM

Retention time: 7.52
Internal standard: pipemidic acid (4.88)
Limit of detection: 250 pg/mL

REFERENCE

Basci,N.E.; Bozkurt,A.; Kalayci,D.; Kayaalp,S.O. Rapid liquid chromatographic assay of ciprofloxacin in human aqueous humor, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 353–356.

SAMPLE

Matrix: aqueous humor, blood
Sample preparation: Aqueous humor. Inject a 10 μL aliquot directly. Plasma. Condition a 3 mL C18 SPE cartridge (Varian) with two 3 mL portions of MeCN and 3 mL buffer. Add 2 mL buffer to 500 μL of plasma, mix, add to the SPE cartridge. Wash with 3 mL buffer. Remove moisture with vacuum (200 mbar) for 10 min. Elute with two 500 μL portions of MeCN:buffer 40:60. Vortex the eluate, inject a 10 μL aliquot. (Buffer was 100 mM Tris adjusted to pH 5.0 with HCl).

HPLC VARIABLES

Column: 300 × 4.6 5 μm endcapped ODS-Hypersil
Mobile phase: MeCN:DMF:10 mM NaH_2PO_4 15:6:79, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 285

CHROMATOGRAM

Retention time: 12.0
Internal standard: ciprofloxacin
Limit of detection: 80 ng/mL (aqueous humor), 310 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: cefotaxime, ofloxacin

KEY WORDS
plasma; SPE; ciprofloxacin is IS

REFERENCE
Kraemer,H.-J.; Gehrke,R.; Breithaupt,A.; Breithaupt,H. Simultaneous quantification of cefotaxime, desacetylcefotaxime, ofloxacine and ciprofloxacine in ocular aqueous humor and in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 147–153.

SAMPLE
Matrix: blood
Sample preparation: Extract plasma sample with dichloromethane:n-butanol 95:5 and dry the solution. Treat organic layer residue with Moscher's acid chloride + triethanolamine in dichloromethane medium for 1 hr. Reconstitute product. Inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5μm ODS
Mobile phase: MeCN:0.2% phosphoric acid 7:3
Column temperature: 35
Detector: F ex 290 em 470

CHROMATOGRAM
Retention time: 7.4
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: grepafloxacin

KEY WORDS
ciprofloxacin is IS; derivatization; plasma

REFERENCE
Tata,P.N.V.; Bramer,S.L. Enantiomeric assay of grepafloxacin in plasma (Abstract 4162), *Pharm.Res.*, **1997**, *14*, S684–S684.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL blood with 1 mL 0.25 mM Triton, vortex for 30 s, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutylammonium hydrogen sulfate adjusted to pH 3.0 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 9
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; pharmacokinetics; rabbit

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: Add 2 mL pH 7.5 phosphate buffer to 250 μL plasma. Extract twice with 5 mL portions of ethyl acetate. Evaporate the organic layer. Reconstitute the residue with mobile phase and inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil C8
Mobile phase: MeCN:2 mM phosphoric acid:triethylamine 15:85:0.15, pH 3.5
Flow rate: 1
Detector: UV 308

CHROMATOGRAM
Retention time: 10
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: sparfloxacin

KEY WORDS
plasma; ciprofloxacin is IS

REFERENCE
Bhatti,M.M.; Hanson,G.D. Determination of cisapride in human plasma by high-performance liquid chromatography with ultraviolet detection (Abstract 2504), *Pharm.Res.*, **1997**, *14*, S378–S378.

SAMPLE
Matrix: blood
Sample preparation: Add 20 μL 10 μg/mL IS in MeOH:0.1% trifluoroacetic acid 15:85 and 5 μL (sic) MeCN to 300 μL plasma. Centrifuge at 600 g for 10 min. Evaporate the supernatant under nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL MeOH: 0.1% trifluoroacetic acid 15:85. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 Zorbax RX-C18
Column: 150 × 4.6 5 μm Zorbax SB-C8
Mobile phase: MeCN:water:trifluoroacetic acid 19:81:0.02
Flow rate: 1
Injection volume: 50
Detector: UV 279

CHROMATOGRAM
Retention time: 4.3-4.4
Internal standard: norfloxacin (3.8-3.9)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: enrofloxacin

KEY WORDS
cat; plasma

REFERENCE

Kordick,D.L.; Papich,M.G.; Breitschwerdt,E.B. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2448–2455.

SAMPLE

Matrix: blood

Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped

Mobile phase: MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)

Column temperature: 37

Flow rate: 1

Detector: UV 279

CHROMATOGRAM

Retention time: 9.71

Internal standard: enoxacin (6.47)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 200 μL 100 mM phosphoric acid, vortex, centrifuge at 3500 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 17 × 4.4 5 μm Nucleosil 100 RP18

Column: 250 × 4.6 5 μm Nucleosil 100 RP18

Mobile phase: Gradient. MeCN:buffer 11:89 for 6.2 min, 50:50 for 3.5 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 50

Flow rate: 1 for 6.2 min, 1.5 for 3.5 min, 1 for 7 min

Detector: F ex 277 em 455

CHROMATOGRAM

Retention time: 6

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; comparison with capillary electrophoresis

REFERENCE
Bannefeld,K.-H.; Stass,H.; Blaschke,G. Capillary electrophoresis with laser-induced fluorescence detection, an adequate alternative to high-performance liquid chromatography, for the determination of ciprofloxacin and its metabolite desethyleneciprofloxacin in human plasma, *J.Chromatogr.B*, **1997**, *692*, 453–459.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Plasma, CSF. Mix 100 μL Plasma or CSF with 1.0 mL 100 mM phosphate buffer. Extract with 5 mL chloroform (Caution! Chloroform is a carcinogen!) containing 1.0% ethyl chloroformate by shaking with a reciprocal shaker for 10 min. Remove 4 mL organic phase, dry it with rotary evaporator at 40°, reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot. Tissue. Homogenize brain sample in 100 mM pH 7.0 phosphate buffer 1:4. Extract 1 mL homogenate with 5 mL dichloromethane by shaking with a reciprocal shaker for 10 min. Remove 4 mL organic phase and back-extract with 4 mL 1 mM sodium hydroxide, extract the aqueous phase as described above for the plasma, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Nucleosil 5C18
Mobile phase: MeOH:5 mM sodium lauryl sulfate 60:40, adjusted to pH 2.5 with phosphoric acid
Column temperature: 50
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: ciprofloxacin
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: enoxacin

KEY WORDS
plasma; brain; rat; derivatization; ciprofloxacin is IS

REFERENCE
Ohtani,H.; Noma,S.; Kawakami,J.; Sawada,Y.; Iga,T. Lack of potentiation with felbinac patch on the convulsive toxicity of enoxacin in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 995–997.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 900 μL 100 mM phosphate buffer, 100 μL 10 μg/mL IS and 5 mL chloroform:ethyl chloroformate 99:1, shake for 10 min, centrifuge at 1620 g for 5 min, evaporate the organic phase under reduced pressure, dissolve the residue in 100 μL MeOH:50 mM NaOH 2:1, inject a 20 μL aliquot. Tissue. Homogenate the cerebrum sample with 4 volumes of 100 mM phosphate buffer. Mix 1 mL homogenate with 100 μL 10 μg/mL IS and 5 mL dichloromethane, shake for 10 min, centrifuge at 1620 g for 5 min. Mix 4 mL 1 mM NaOH with 4 mL organic phase, shake for 10 min, centrifuge at 1620 g for 5 min, collect 3 mL aqueous phase and treat in a manner similar to that for the plasma sample, except for the IS addition. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5 C18
Mobile phase: MeOH:5 mM sodium dodecylsulfate adjusted to pH 2.5 with phosphoric acid

Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: pipemidic acid

OTHER SUBSTANCES
Extracted: foscarnet
Simultaneous: enoxacin

KEY WORDS
plasma; brain; mouse; pharmacokinetics; derivatization

REFERENCE
Matsuo,H.; Ryu,M.; Nagata,A.; Uchida,T.; Kawakami,J.-I.; Yamamoto,K.; Iga,T.; Sawada,Y. Neurotoxicodynamics of the interaction between ciprofloxacin and foscarnet in mice, *Antimicrob.Agents Chemother.*, **1998**, *42*, 691–694.

SAMPLE
Matrix: blood, urine
Sample preparation: Mix 250 μL plasma or 10 μL urine with 250 μL phosphate buffer Add 4 mL dichloromethane, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot. (Phosphate buffer was 70 mM KH_2PO_4:80 mM Na_2HPO_4 40:60.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeOH:5 mM copper (II) sulfate pentahydrate containing 10 mM L-isoleucine 12.5:87.5
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 330

CHROMATOGRAM
Retention time: 13
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: levofloxacin

KEY WORDS
plasma; ciprofloxacin is IS

REFERENCE
Wong,F.A.; Juzwin,S.J.; Flor,S.C. Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 765–771.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. 500 μL Serum or plasma + 100 μL 20 μg/mL IS in 100 mM phosphoric acid + 300 μL MeCN:5 M trichloroacetic acid 50:50, vortex, add 100 μL MeCN, add 300 μL water, vortex, centrifuge at 1500 g for 15 min, inject a 10 μL aliquot of the supernatant. Urine. Dilute urine 1:20 (or more) with 50 mM pH 3.0 KH_2PO_4, remove a

500 μL aliquot and add it to 100 μL 20 μg/mL IS in 100 mM phosphoric acid, add 700 μL 100 mM trichloroacetic acid, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S (Polymer Laboratories)
Column: 150 × 4.6 PLRP-S (Polymer Laboratories)
Mobile phase: MeCN:MeOH:20 mM pH 3.0 trichloroacetic acid 22:4:74
Column temperature: 30
Flow rate: 0.7
Injection volume: 10
Detector: F ex 277 em 418 following post-column photolysis. The column effluent flowed through a 10 m × 0.25 mm knitted PTFE coil irradiated with a UV 254 low pressure lamp and flowed to the detector.

CHROMATOGRAM
Retention time: 8
Internal standard: 1-isopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (13)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Krol,G.J.; Beck,G.W.; Benham,T. HPLC analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 181–190.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 278.3

CHROMATOGRAM
Retention time: 9.102

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve a sample in mobile phase, sonicate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:THF:buffer 5:10:85 (Buffer was 5 mM 1-hexanesulfonic acid adjusted to pH 3.0 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11.5-15.0
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Lacroix,P.M.; Curran,N.M.; Sears,R.W. High-performance liquid chromatographic methods for ciprofloxacin hydrochloride and related compounds in raw materials, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 641–654.

SAMPLE
Matrix: growth medium
Sample preparation: 500 μL Growth medium + 500 μL 100 μg/mL IS in cold (4°) MeCN, vortex, centrifuge at 3000 g for 5 min. Remove a 500 μL aliquot of the supernatant, filter (0.45 μm Acrodisc syringe filter), inject a 30 μL aliquot. (Protect all specimens from light.)

HPLC VARIABLES
Guard column: C18 5U (Alltech)
Column: 150 × 4.6 7 μm Adsorbosphere HS C18 7U
Mobile phase: MeCN:20 mM pH 3.0 phosphate buffer 35:65 containing 0.2% triethylamine and 0.2% sodium dodecyl sulfate, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1.75
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 4.67
Internal standard: sparfloxacin (7.09)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: clinafloxacin, levofloxacin, ofloxacin, sparfloxacin, temafloxacin, trovafloxacin

KEY WORDS
Mueller-Hinton broth

REFERENCE

Wright,D.H.; Herman,V.K.; Konstantinides,F.N.; Rotschafer,J.C. Determination of quinolone antibiotics in growth media by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *709*, 97–104.

SAMPLE

Matrix: milk

Sample preparation: Condition a 500 mg Bond Elut LRC PRS SPE cartridge with 5 mL MeOH and 5 mL extracting solution 65:35. Add 25 mL extracting solution to 5 mL milk, shake for 15 s, add 4 g anhydrous sodium sulfate, shake for 15 s, centrifuge at 3000 rpm at 5° for 5 min. Remove the supernatant and repeat the extraction with 25 mL extracting solution as before except do not add any more sodium sulfate, mix mechanically, centrifuge, combine the supernatants, add 25 mL 1% acetic acid, shake for 10-15 s. Freeze for 30 min to facilitate precipitation, centrifuge at 2500 rpm at 5° for 10 min. Add 75 mL to the SPE cartridge, pass the entire sample through the cartridge, then add 2 mL MeOH, wash with 5 mL water, wash with 2 mL MeOH. Elute with 2.5 mL 25% ammonium hydroxide-MeOH. Evaporate to dryness under nitrogen at 55°, dissolve the residue in 2 mL 1% acetic acid, sonicate for 1 min, vortex for 20 s, filter (0.45 μm), inject an aliquot. (Extracting solution was 1% aqueous acetic acid:EtOH 1:99.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil
Mobile phase: MeCN:2% acetic acid 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 450, with a 418 nm cut-off filter

CHROMATOGRAM

Retention time: 3.1
Limit of detection: 0.4 ppb
Limit of quantitation: 5 ppb

OTHER SUBSTANCES

Extracted: enrofloxacin, difloxacin, sarafloxacin

KEY WORDS

SPE

REFERENCE

Roybal,J.E.; Pfenning,A.P.; Turnipseed,S.B.; Walker,C.C.; Hurlbut,J.A. Determination of four fluoroquinolones in milk by liquid chromatography, *J.AOAC Int.*, **1997**, *80*, 982–987.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Asahipack ODP 50
Mobile phase: MeCN:water from 10:90 to 40:60 over 10 min
Detector: UV

OTHER SUBSTANCES

Simultaneous: sparfloxacin

REFERENCE

Rispal,P.; Grellet,J.; Celerier,C.; Breilh,D.; Dorian,M.; Pellegrin,J.L.; Saux,M.C.; Leng,B. Comparative uptake of sparfloxacin and ciprofloxacin into human THP 1 monocytic cells, *Arzneimittelforschung*, **1996**, *46*, 316–319.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 4 μm NovaPak C18
Mobile phase: MeCN:MeOH:buffer:acetic acid 2.5:10:86.5:1 containing 20 mM triethylamine (The pH 2.7 buffer was 0.4% diammonium hydrogen phosphate in water containing 0.4% (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Detector: UV 279

CHROMATOGRAM
Retention time: 21.6

OTHER SUBSTANCES
Extracted: enrofloxacin, ofloxacin

REFERENCE
Cester,C.C.; Toutain,P.L. A comprehensive model for enrofloxacin to ciprofloxacin transformation and disposition in dog, *J.Pharm.Sci.*, **1997**, *86*, 1148–1155.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: enoxacin, fleroxacin, norfloxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography. Prediction of retention of a series of quinolones, *J.Chromatogr.A*, **1996**, *719*, 27–36.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC PRS SPE cartridge with 2 mL MeOH and 2 mL equilibrating solution. 2 g Catfish muscle + 18 mL extracting solution, homogenize for 20 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add another 18 mL extracting solution to the pellet and homogenize again, centrifuge at 3000 rpm for 5 min, combine the supernatants. Add 20 mL 1% glacial acetic acid, freeze for 30 min, centrifuge at 2500 rpm at 4° for 10 min. Add the extracts to the SPE cartridge, wash with 2 mL MeOH, 5 mL water, and 2 mL MeOH. Let the SPE cartridge dry for 30 s. Elute with 2 mL MeOH:30% ammonium hydroxide 80:20, dry the eluate under nitrogen at 50°. Reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject an aliquot.

(The extracting solution was EtOH:water:glacial acetic acid 98:1:1. The equilibrating solution was extracting solution:1% glacial acetic acid 35:20.)

HPLC VARIABLES

Column: 150 × 2 5 μm Inertsil Phenyl
Mobile phase: MeCN:2% formic acid 14:86
Column temperature: 40
Flow rate: 0.35
Injection volume: 50
Detector: MS, Hewlett-Packard 5989, Model 59987A electrospray, nitrogen drying gas 40 mL/min, 260°, nebulizing gas nitrogen, 80 psi, m/z 231

CHROMATOGRAM

Retention time: 3.74-3.84
Limit of detection: 10 ppb
Limit of quantitation: 20 ppb

OTHER SUBSTANCES

Extracted: enrofloxacin

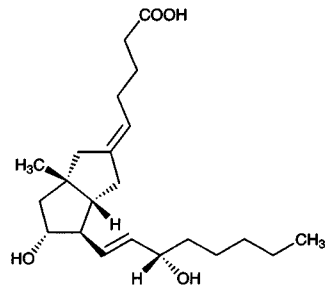
KEY WORDS

catfish; muscle; SPE

REFERENCE

Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Pfenning,A.P.; Hurlbut,J.A. Confirmation of fluoroquinolones in catfish muscle by electrospray liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1998**, *81*, 554–562.

Ciprostene



Molecular formula: $C_{22}H_{36}O_8$
Molecular weight: 727.04
CAS Registry No.: 81845-44-5, 81703-55-1 (calcium salt)
Lednicer No.: 4 14

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg Bond Elut C2 SPE cartridge with two 1 mL portions of MeCN, with 1 mL water, and with 1 mL 1% phosphoric acid, do not allow to dry. Condition a Bond Elut CN SPE cartridge with two 1 mL portions of n-hexane. 250 μL Plasma + 50 μL 375 ng/mL carbacyclin in MeOH + 750 μL 1% phosphoric acid, add to the C2 SPE cartridge, wash with two 1 mL portions of water, wash with two 1 mL portions of MeOH:water 40:60, dry under vacuum for 10 min, wash with two 1 mL portions of n-hexane:MTBE 75:25, elute with 1 mL n-hexane:MTBE 20:80. Evaporate the eluate to dryness under reduced pressure, reconstitute with 100 μL MeOH, evaporate to dryness under a stream of air, add 10 mg solid potassium bicarbonate:sodium sulfate 1:1, add 50 μL 200 μM dibenzo-18-crown-6 in acetone, add 50 μL 1 mM 4-bromomethyl-7-acetoxycoumarin in acetone, stir at 50° for 30 min, cool, add 900 μL n-hexane, vortex, add to the CN SPE cartridge, wash with two 1 mL portions of n-hexane, wash with two 1 mL portions of n-hexane:ethyl acetate 80:20, dry under vacuum for 1 min, elute slowly with 1 mL MeCN. Evaporate the eluate to dryness under a stream of air at 30°, reconstitute the residue in 50 μL MeCN:water:trifluoroacetic acid 50:50:0.1, inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 NewGuard RP18
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:water:trifluoroacetic acid 55:44.9:0.1
Column temperature: 45
Flow rate: 1.5
Detector: F ex 370 em 466 following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.5 mL/min and the mixture flowed through a 4 mL knitted coil of PTFE tubing at 80° to the detector.

CHROMATOGRAM

Retention time: 31.3
Internal standard: carbacyclin (23.7)
Limit of quantitation: 5 ng/mL

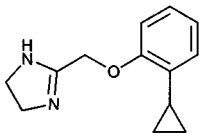
KEY WORDS

plasma; derivatization; post-column reaction; SPE

REFERENCE

James,C.A.; Simmonds,R.J.; Burton,N.K. An HPLC assay for a prostacyclin analogue, ciprostene calcium, in human plasma, *J.Liq.Chromatogr.*, **1990**, *13*, 1143–1158.

Cirazoline



Molecular formula: $C_{13}H_{16}N_2O$
Molecular weight: 216.28
CAS Registry No.: 59939-16-1

SAMPLE
Matrix: solutions

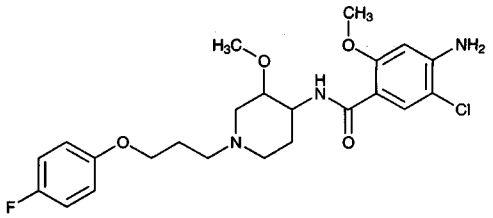
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.71

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Cisapride



Molecular formula: $C_{23}H_{29}ClFN_3O_4$
Molecular weight: 465.95
CAS Registry No.: 81098-60-4
Merck Index: 2377

SAMPLE

Matrix: blood
Sample preparation: Add IS to 1 mL plasma, extract under basic conditions with 5 mL n-butyl chloride. Evaporate the organic layer to dryness under nitrogen. Dissolve the residue in 200 μL mobile phase and inject an aliquot.

HPLC VARIABLES

Column: 150 × 2 Hypersil-ODS
Mobile phase: MeCN:water:triethylamine 37:62.9:0.1
Detector: UV 276

CHROMATOGRAM

Retention time: 4.6
Internal standard: R054680 (10.1)
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma

REFERENCE

Sirisuth,N.; Joubert,A.E.; Eddington,N.D. Development and validation of an HPLC method for ADD 196022, a member of the new class of enaminone anticonvulsants (Abstract 2496), *Pharm.Res.*, **1997**, *14*, S376–S376.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM

Retention time: 14.627

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cisplatin

Molecular formula: $Cl_2H_6N_2Pt$
Molecular weight: 300.05
CAS Registry No.: 15663-27-1
Merck Index: 2378
Lednicer No.: 4 15-17

SAMPLE

Matrix: blood
Sample preparation: Filter (Amicon Centriflo CF 50A) while centrifuging at 2500 rpm, inject a 25 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: Guard-Pak C18 (Waters)
Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: 10 mM pH 4.6 Sodium acetate buffer containing 5 mM heptanesulfonic acid
Flow rate: 1 (A), 0.5 (B, C)
Injection volume: 25
Detector: E, HMDE 174 A/310 (EG & G, Princeton Applied Research), hanging mercury drop electrode, polarographic cell, operated at 100 mV vs Ag/AgCl in HMDE mode with small drop size (A); E, LC4B (Bioanalytical Systems, West Lafayette IN) with a Model TL-9A thin-layer transducer cell, Au/Hg amalgam working electrode at -100 mV, reference electrode Ag/AgCl (B); atomic absorption of fractions, IL 951-555 systems (Allied Analytical Systems, Lexington MA), 265.95 nm, band-pass 0.5 nm, lamp current 10 mA (C)

CHROMATOGRAM

Retention time: 1.6
Limit of detection: 62 ng/mL

KEY WORDS

plasma; ultrafiltrate; protect MP from oxygen; comparison thin-layer Au/Hg electrode and HMDE

REFERENCE

Parsons,P.J.; LeRoy,A.F. Determination of cis-diamminedichloroplatinum(II) in human plasma using ion-pair chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *378*, 395–408.

SAMPLE

Matrix: blood
Sample preparation: Inject an aliquot of plasma ultrafiltrate.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil ODS coated with hexadecyltrimethylammonium bromide (Coat the column by passing 75 mL of a 27 mM aqueous solution of hexadecyltrimethylammonium bromide through the column at 30° at 1 mL/min (J.Chromatogr. 1981, 217, 405).)
Mobile phase: 10 mM pH 4.5 Citrate buffer containing 100 μM hexadecyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 290 following post-column reaction. The column effluent mixed with 26 μM potassium dichromate pumped at 0.1 mL/min and this mixture flowed through a 3.2 m × 0.3 mm i.d. knitted coil of PTFE tubing. The effluent from the coil mixed with 3.3 mM

sodium bisulfite solution pumped at 0.1 mL/min and this mixture flowed through a 44.6 m × 0.3 mm i.d. knitted coil of 0.3 mm i.d. PTFE tubing to the detector.

CHROMATOGRAM
Retention time: 9
Limit of detection: 40 ng/mL

KEY WORDS
plasma; ultrafiltrate; post-column reaction

REFERENCE
Marsh,K.C.; Sternson,L.A.; Repta,A.J. Post-column reaction detector for platinum(II) antineoplastic agents, *Anal.Chem.*, **1984**, *56*, 491–497.

SAMPLE
Matrix: blood
Sample preparation: Filter through a 10000 molecular mass cut-off filter (Filtron) by centrifuging at 4000 g at 4° for 30 min, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil SB strong anion exchanger
Mobile phase: MeOH:125 mM succinic acid adjusted to pH 5.2 with NaOH 60:40
Flow rate: 0.5
Detector: UV 344 following post-column derivatization with 20 mM sodium diethyldithiocarbamate in MeOH:water 60:40 pumped at 0.17 mL/min. The mixture flowed through a 500 × 3.5 mm packed-bed reactor packed with 75 μm silanized glass beads at 115° then to the detector.

CHROMATOGRAM
Retention time: 13
Limit of detection: about 200 ng/mL

KEY WORDS
plasma; human; guinea pig; post-column reaction

REFERENCE
Andersson,A.; Ehrsson,H. Determination of cisplatin and cis-diammineaquachloroplatinum(II) ion by liquid chromatography using post-column derivatization with diethyldithiocarbamate, *J.Chromatogr.B*, **1994**, *652*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 400 μL MeCN, mix, centrifuge at 4° at 3500 g for 5 min. Remove a 200 μL aliquot of the supernatant and add it to 100 μL 10 mM citric acid containing 100 μM cetyltrimethylammonium bromide and 700 μL dichloromethane, rotate for 10 min, centrifuge at 4° at 3500 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Polymer RP (Brownlee)
Column: 100 × 4.6 BDS-Hypersil C18 (Before analyses pump 120 mL 50 mM cetyltrimethylammonium bromide in isopropanol:water 5:95 through the column at 30° to coat the column with cetyltrimethylammonium bromide.)
Mobile phase: 10 mM Citric acid containing 100 μM cetyltrimethylammonium bromide, adjusted to pH 5.0 with 5 M NaOH
Column temperature: 25
Flow rate: 0.7

Injection volume: 40
Detector: UV 290 following post-column reaction. The column effluent mixed with 117 μM potassium dichromate pumped at 0.2 mL/min and the mixture flowed through a 200 μL knitted coil of PTFE tubing at 30° and then mixed with 28.16 mM sodium bisulfite pumped at 0.2 mL/min. This mixture flowed through a 1 mL knitted coil of PTFE tubing at 30° to the detector. (The reaction coils were contained in a PCX 5000 Post Column Reaction Module (Pickering Laboratories, Mountain View CA).)

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 60 ng/mL

KEY WORDS
post-column reaction; plasma; dog; pharmacokinetics

REFERENCE
Farrish,H.H.; Hsyu,P.-H.; Pritchard,J.F.; Brouwer,K.R.; Jarrett,J. Validation of a liquid chromatography post-column derivatization assay for the determination of cisplatin in plasma, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 265–271.

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon 14 mm YMT-1 membrane) 1 mL plasma while centrifuging at 2000 g for 45 min. 500 μL Ultrafiltrate + 30 μL 100 μg/mL nickel chloride in 0.9% NaCl solution + 50 μL 10% sodium diethyldithiocarbamate in 100 mM NaOH (freshly prepared), heat at 37° for 1 h, cool, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Hypersil ODS C18
Column: 250 × 4.6 5 μm Ultrasphere
Mobile phase: MeOH:water 75:25
Column temperature: 30
Flow rate: 1.5
Injection volume: 100
Detector: UV 260 for 6.5 min then UV 250

CHROMATOGRAM
Retention time: 5.7
Internal standard: nickel chloride (7.4)
Limit of detection: 10 ng/mL
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Noninterfering: calcium, cobalt, copper, cyclophosphamide, etoposide, 5-fluorouracil, iron, lead, mitomycin, phosphorus, vinorelbine

KEY WORDS
plasma; ultrafiltrate; method does not distinguish between cisplatin and platinum-containing metabolites

REFERENCE
Augey,V.; Cociglio,M.; Galtier,M.; Yearoo,R.; Pinsani,V.; Bressolle,F. High-performance liquid chromatographic determination of cis-dichlorodiammineplatinum(II) in plasma ultrafiltrate, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1173–1178.

SAMPLE
Matrix: blood

Sample preparation: Filter (Amicon centriflow ultrafiltration membrane CF50, cut-off 50 000 Da) plasma while centrifuging at 4° at 1000 g for 10 min, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP18
Mobile phase: 15 mM pH 2.2 phosphoric acid
Flow rate: 1
Injection volume: 10-20
Detector: atomic absorption (Collect 15 s fractions of the HPLC effluent, analyze a 20 μL aliquot by atomic absorption with graphite furnace and deuterium background correction (Varian Model SpectrAA 300).)

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

rat; human; plasma; ultrafiltrate

REFERENCE

Bernareggi,A.; Torti,L.; Maffei Facino,R.; Carini,M.; Depta,G.; Casetta,B.; Farrell,N.; Spadacini,S.; Ceserani,R.; Tognella,S. Characterization of cisplatin-glutathione adducts by liquid chromatography-mass spectrometry. Evidence for their formation in vitro but not in vivo after concomitant administration of cisplatin and glutathione to rats and cancer patients, *J.Chromatogr.B*, **1995**, *669*, 247–263.

SAMPLE

Matrix: blood, formulations
Sample preparation: Whole blood. 5 mL Whole blood + 2 mL 10% trichloroacetic acid in water, mix thoroughly, let stand for 15 min, centrifuge for 20 min. Remove the supernatant and add it to 2 mL concentrated HCl, add 8 mL 10% trichloroacetic acid in water, mix, centrifuge for 15 min. Remove the supernatant and add it to 2 mL concentrated HCl, evaporate to near dryness with heat, dissolve the residue in 3 mL water, adjust the pH to 6, add 2 mL pH 8 sodium bicarbonate buffer, add 2 mL 1% bis(salicaldehyde)tetramethylethylenediamine in EtOH, warm for 15 min, cool, add 4 mL chloroform, mix well. Remove the chloroform layer and evaporate it to dryness, reconstitute with 100 μL MeOH, inject a 5 μL aliquot. Injections. Add 1 g cisplatin injection to 30 mL concentrated HCl, evaporate to dryness with heat, reconstitute with 10 mL water. Remove a 5 mL aliquot, add 2 mL pH 8 sodium bicarbonate buffer, add 2 mL 1% bis(salicaldehyde)tetramethylethylenediamine in EtOH, warm for 15 min, cool, add 4 mL chloroform, mix well. Remove a 2 mL aliquot of the chloroform layer and evaporate it to dryness, reconstitute with 1 mL EtOH, inject a 5 μL aliquot. (Preparation of bis(salicaldehyde)tetramethylethylenediamine is as follows. Stir 44.5 g 2-nitropropane and 84 mL 6 M NaOH with cooling, add 40 g bromine dropwise, add 165 mL EtOH, reflux gently for 3 h, pour into 500 mL ice-water, filter to obtain 2,3-dimethyl-2,3-dinitrobutane. Vigorously stir 17.6 2,3-dimethyl-2,3-dinitrobutane with 150 mL concentrated HCl at 50-60°, slowly add 75 g 20-mesh granular tin, reflux for 15 min, cool, make strongly alkaline with NaOH (Caution! Exothermic!), steam distil, collect 350 mL distillate. Add 100 g solid NaOH to the distillate to obtain 2,3-diamino-2,3-dimethylbutane as a separate layer (mp of oxalate 323-324°) (J. Am. Chem. Soc. 1955, 77, 6689). Stir 20 mmoles salicylaldehyde in 10 mL MeOH, add 10 mmoles 2,3-diamino-2,3-dimethylbutane in 6 mL MeOH dropwise, let stand for several h, collect the precipitate, recrystallize twice from MeOH to obtain bis(salicaldehyde)tetramethylethylenediamine as yellow needles (mp 117°) (Inorg. Chem. 1978, 7, 3389).)

HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 30:50:20
Flow rate: 0.4
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: copper(II), iron(II), nickel(II), palladium(II), uranyl

KEY WORDS
derivatization; complexation; injections; whole blood

REFERENCE
Khuhawar,M.Y.; Lanjwani,S.N.; Memon,S.A. High-performance liquid chromatographic determination of cisplatin as platinum(II) in a pharmaceutical preparation and blood samples of cancer patients, *J.Chromatogr.B*, **1997**, *693*, 175–179.

SAMPLE
Matrix: blood, urine
Sample preparation: Filter using a UFC 3GC membrane with a 10000 molecular weight cut-off (Japan Millipore) at 4000 g at 4° for 30 min, inject a 100 μL aliquot of the ultrafiltrate. Urine. Centrifuge at 1000 g for 1 min, dilute a 50 μL aliquot of the upper layer 1:10 with distilled water, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Cyano Guard-Pak (Waters)
Column: 150 × 4.6 5 μm anionic exchange resin (Hitachi No. 3013-N, Chromato Research)
Mobile phase: MeCN:10 mM NaCl 85:15
Column temperature: 40
Flow rate: 0.7
Injection volume: 100
Detector: UV 290 following post-column derivatization with 0.026 mM potassium dichromate at 0.14 mL/min and 6.6 mM sodium hydrogen sulfite at 0.07 mL/min using a 7000 × 0.5 mm or 30000 × 0.25 mm PTFE tube reactor.

CHROMATOGRAM
Retention time: 11
Limit of detection: 80 ng/mL

KEY WORDS
plasma; post-column reaction

REFERENCE
Kinoshita,M.; Yoshimura,N.; Ogata,H.; Tsujino,D.; Takahashi,T.; Takahashi,S.; Wada,Y.; Someya,K.; Ohno,T.; Masuhara,K.; Tanaka,Y. High-performance liquid chromatographic analysis of unchanged cis-diamminedichloroplatinum (cisplatin) in plasma and urine with post-column derivatization, *J.Chromatogr.*, **1990**, *529*, 462–467.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Direct injection. Plasma. Filter through a 10000 molecular mass cut-off membrane, inject an aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 5 μm Spherisorb ODS-2
Column: 150 × 4.6 5 μm OD5 C18 (Burdick & Jackson)
Mobile phase: 5 mM Sodium heptanesulfonate, 10% MeOH, 0.1% trifluoroacetic acid, pH 2.6
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: MS, Sciex Elan 250 ICP-MS coupled to the column with 960 × 0.1 mm PTFE capillary tubing, m/z 195 monitored, RF power 1.25 kW, nebulizer argon 0.9 L/min, auxiliary argon 1.2 L/min, coolant 12.5 L/min, spray chamber 5°.

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, hydrolysis products

KEY WORDS

plasma

REFERENCE

Zhao,Z.; Tepperman,K.; Dorsey,J.G.; Elder,R.C. Determination of cisplatin and some possible metabolites by ion-pairing chromatography with inductively coupled plasma mass spectrometric detection, *J.Chromatogr.*, **1993**, *615*, 83–89.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon MPS-1 with a YMT membrane) while centrifuging at 3000 g at 4° for 15 min, inject an aliquot of the ultrafiltrate. Urine. Inject an aliquot directly.

HPLC VARIABLES

Column: 80 × 4.6 MCI gel CDR10
Mobile phase: MeCN:buffer 30:70 (Buffer was 100 mM sodium sulfate and 10 mM acetate, pH 5.5.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 290 following post-column derivatization. The column effluent mixed with the reagent pumped at 0.3 mL/min, the mixture flowed through a 10 m × 0.5 mm i.d. coil of PTFE tubing held at 60° to the detector. The reagent was 40 mM sodium bisulfite and 10 mM acetate buffer, pH 5.5.

CHROMATOGRAM

Retention time: 10
Limit of detection: 20 nM

KEY WORDS

plasma; ultrafiltrate; rabbit; human; post-column reaction

REFERENCE

Kizu,R.; Yamamoto,T.; Yokoyama,T.; Tanaka,M.; Miyazaki,M. A sensitive postcolumn derivatization/UV detection system for HPLC determination of antitumor divalent and quadrivalent platinum complexes, *Chem.Pharm.Bull.*, **1995**, *43*, 108–114.

SAMPLE
Matrix: formulations
Sample preparation: Adjust pH to 7.0, dilute if necessary, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.2 5 μm Nucleosil C18
Mobile phase: 10 mM pH 7.0 phosphate buffer containing 0.55 mM hexadecyltrimethylammonium bromide (Condition column before use with 0.5% hexadecyltrimethylammonium bromide.)
Flow rate: 1
Detector: UV 216

CHROMATOGRAM
Retention time: 7
Limit of detection: 1000 ng/mL
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Simultaneous: carboplatin

KEY WORDS
infusions; stability-indicating

REFERENCE
Rochard,E.; Boutelet,H.; Griesemann,E.; Barthes,D.; Courtois,P. Simultaneous high performance liquid chromatographic analysis of carboplatin and cisplatin in infusion fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 1505–1516.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 1.90

OTHER SUBSTANCES
Simultaneous: cimetidine (UV 228), dacarbazine (UV 300), granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

Sample preparation: Mix 2 mL of a 750 μg/mL solution of cisplatin in water with 1 mL 1.8 mg/mL o-phenylenediamine in water, heat at 100° for 20 min, filter (paper) the precipitate and wash it with water. Dissolve the precipitate in DMF and inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: Chloroform
Flow rate: 1
Injection volume: 100
Detector: UV 703

CHROMATOGRAM
Retention time: 3
Limit of detection: 400 ng/mL Pt

KEY WORDS
derivatization; complexation

REFERENCE
Hasson,H.; Warshawsky,A. High performance liquid chromatographic determination of *cis*-diammine-dichloroplatinum(II) (cisplatin) as the *o*-phenylenediamine complex, *J.Chromatogr.*, **1990**, *530*, 219–221.

SAMPLE
Matrix: solutions
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 250 × 4 Silasorb SPH C18
Mobile phase: 4 mM sodium octanesulfonate + 6 mM tetrabutylammonium hydrogen sulfate + 20 mM KH_2PO_4, pH adjusted with concentrated NaOH to 5.9
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Macka,M.; Borák,J.; Semenková,L.; Kiss,F. Decomposition of cisplatin in aqueous solutions containing chlorides by ultrasonic energy and light, *J.Pharm.Sci.*, **1994**, *83*, 815–818.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 100 × 4.6 7 μm Hypercarb S porous graphitic carbon (Shandon)
Mobile phase: 1 mM NaOH
Column temperature: 0, 20
Flow rate: 0.5
Injection volume: 100

Detector: UV 283

CHROMATOGRAM
Retention time: 8.5 (0°), 6.5 (20°)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Ehrsson,H.C.; Wallin,I.B.; Andersson,A.S.; Edlund,P.O. Cisplatin, transplatin, and their hydrated complexes: Separation and identification using porous graphitic carbon and electrospray ionization mass spectrometry, *Anal.Chem.*, **1995**, *67*, 3608–3611.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize with four volumes saline at 4°, centrifuge at 100000 g at 4° for 1 h. Remove a 450 μL aliquot of the supernatant, filter (Millipore UFC 3GC 10000 molecular mass cut-off) with centrifuging at 4000 g at 4° for 30 min, inject a 20 μL aliquot of the supernatant

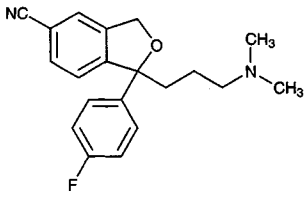
HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Hitachi No. 3013-N
Column: 150 × 4.6 5 μm Hitachi No. 3013-N
Mobile phase: MeCN:10 mM NaCl 15:85
Column temperature: 40
Flow rate: 0.9
Injection volume: 100
Detector: UV 290 following post-column derivatization with 0.026 mM potassium dichromate at 0.6 mL/min and 6.6 mM sodium hydrogen sulfite at 0.3 mL/min using a 7000 × 0.5 mm or 30000 × 0.25 mm PTFE tube reactor.

CHROMATOGRAM
Retention time: 9
Limit of detection: 100 ng/g

KEY WORDS
rat; liver; kidney; ultrafiltrate; pharmacokinetics; post-column reaction; derivatization

REFERENCE
Hanada,K.; Nagai,N.; Ogata,H. Quantitative determination of unchanged cisplatin in rat kidney and liver by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 181–186.

Citalopram



Molecular formula: $C_{20}H_{21}FN_2O$
Molecular weight: 324.40
CAS Registry No.: 59729-33-8, 59729-32-7 (HBr)
Merck Index: 2379

SAMPLE

Matrix: blood
Sample preparation: Condition a 10 mL 100 mg Isolute C2 SPE cartridge (International Sorbent Technology) with 1 mL MeCN and 500 μL 25 mM pH 11.5 phosphate buffer. Add 1 mL MeCN:25 mM pH 11.5 phosphate buffer 5:95, 1 mL plasma, 100 μL 500 ng/mL IS, and 1 mL MeCN:25 mM pH 11.5 phosphate buffer 5:95 to the SPE cartridge reservoir. Suck the solution through the cartridge (15-35 kPa). Wash with 1 mL water, dry under vacuum for 5 min, wash with 1 mL MeCN, dry. Add 2 mL 1% acetic acid in MeOH, leave for 5 min, draw solvent through cartridge with vacuum (15-35 kPa). Evaporate eluate with nitrogen at 70°. Reconstitute with 100 μL mobile phase. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 μm Nova Pak phenyl
Column: 100 × 8 4 μm Nova Pak phenyl RCM radial compression
Mobile phase: MeCN:70 mmol/L phosphate buffer 40:60
Flow rate: 3
Injection volume: 20
Detector: F ex 235 em 290

CHROMATOGRAM

Retention time: 3.9
Internal standard: LU 10-202-O (5.8)
Limit of detection: 800 pM

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: alprazolam, amitriptyline, buspirone, clomipramine, clozapine, diazepam, desipramine, desmethylclomipramine, flunitrazepam, flupenthixol, haloperidol, hydroxyzine, imipramine, levopromazine, lofepramine, maprotiline, mianserin, nitrazepam, nortriptyline, oxazepam, paroxetine, perphenazine, propiomazin, thioridazine, zolpidem

KEY WORDS

plasma; SPE

REFERENCE

Carlsson,B.; Norlander,B. Solid-phase extraction with end-capped C2 columns for the routine measurement of racemic citalopram and metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 234–239.

SAMPLE

Matrix: blood
Sample preparation: Take 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:55:10
Flow rate: 0.6
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.8
Internal standard: citalopram

OTHER SUBSTANCES
Simultaneous: metapramine
Noninterfering: indalpine, diazepam, amitriptyline, clobazam, levomepromazine, norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine
Interfering: carbamazepine

KEY WORDS
plasma; citalopram is IS

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: 1-2 mL Plasma + 50 μL 10 μg/mL desipramine in MeOH, make up to 3 mL with 100 mM NaOH, vortex for 1 min, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL diethyl ether. Add the eluate to 50 μL 5 mM phosphoric acid, evaporate under a stream of air at 40°. Add 1 mL diethyl ether to the residual solution, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak C18 guard-pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 41:50:9
Flow rate: 0.8
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Retention time: 11.5
Internal standard: desipramine (14.5)
Limit of detection: 0.8 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: metoclopramide, oxazepam, dihydroergotamine, lorazepam, bromazepam, methotrimeprazine, cisapride, clobazam, diazepam, cyamemazine, alimemazine
Noninterfering: heptaminol, meprobamate, caffeine

KEY WORDS
plasma; SPE

REFERENCE

Rop,P.P.; Durand,A.; Viala,A.; Jorgensen,A. Simultaneous determination of citalopram, monodesmethylcitalopram and didesmethylcitalopram in plasma by high-performance liquid chromatography after column extraction, *J.Chromatogr.*, **1990**, *527*, 226–232.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 2 μL 10 μg/mL IS in water, inject on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A onto column B with mobile phase B, after 2 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 4 × 4 LiChrospher 100 RP-18; B 150 × 4.6 Ultron N-C18 (Shinwa Chemical Industries)

Mobile phase: A 1 mM pH 3.0 phosphate buffer; B MeCN:20 mM pH 4.6 phosphate buffer 30:70 containing 0.1% diethylamine

Column temperature: 40 (column B)

Flow rate: 1

Injection volume: 200

Detector: F ex 249 em 302

CHROMATOGRAM

Retention time: 20

Internal standard: 1-[3-(dimethylamino)propyl]-1-(4-chlorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile oxalate (H. Lundbeck Lu 10-202) (29)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

column-switching; plasma; dog; rat; pharmacokinetics

REFERENCE

Matsui,E.; Hoshino,M.; Matsui,A.; Okahira,A. Simultaneous determination of citalopram and its metabolites by high-performance liquid chromatography with column switching and fluorescence detection by direct plasma injection, *J.Chromatogr.B*, **1995**, *668*, 299–307.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 1 μg/mL benzoctamine in 10 mM HCl + 100 μL 1 μg/mL desmethylbenzoctamine in 10 mM HCl + 50 μL 1 M NaOH + 6 mL heptane:isoamyl alcohol 98.5:1.5, shake for 15 min, centrifuge at 8° at 2100 g for 6 min. Remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 15 min, centrifuge at 8° at 2100 g for 15 min. Discard the organic phase and add 1 mL 1 M pH 9.4 sodium carbonate buffer to the aqueous phase, add 150 μL toluene:isoamyl alcohol 85:15, vortex for 15 min, centrifuge at 8° at 2100 g for 3 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 60 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm acetylated β-Cyclobond (Astec)

Mobile phase: MeCN:1% diethylamine 22:78 adjusted to pH 6.1 with acetic acid

Flow rate: 0.8

Injection volume: 50

Detector: F ex 240 em 296

CHROMATOGRAM
Retention time: 14.6 (S-(+)), 15.8 (R-(-))
Internal standard: benzoctamine (7.3), desmethylbenzoctamine (6.5)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral

REFERENCE
Rochat,B.; Amey,M.; Baumann,P. Analysis of enantiomers of citalopram and its demethylated metabolites in plasma of depressive patients using chiral reverse-phase liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 273–279.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 100 μL 1-2 μg/mL desipramine + 1 mL 1 M NaOH + 250 mg NaCl + 6 mL dichloromethane, shake for 15 min, centrifuge at 1500 g for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 100-150 μL aliquot. Tissue. Homogenize brain tissue gently (Ultra-Turrax) with two volumes 0.9% saline. 1 mL Homogenate + 100 μL 1-2 μg/mL desipramine + 1 mL 1 M NaOH + 250 mg NaCl + 6 mL dichloromethane, shake for 15 min, centrifuge at 1500 g for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 100-150 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm Spherisorb ODS C18
Mobile phase: MeCN:25 mM KH_2PO_4 50:50
Flow rate: 1.5
Injection volume: 100-150
Detector: UV 240

CHROMATOGRAM
Retention time: 9.3
Internal standard: desipramine (12.2)
Limit of detection: 25 ng/mL

KEY WORDS
plasma; rat; brain

REFERENCE
Wang,N.-S.; Lemmer,B. Determination of citalopram in plasma and brain tissue of the rat by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *488*, 492–497.

SAMPLE
Matrix: serum
Sample preparation: 1 mL Serum + 500 μL 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μL IS in EtOH:water 50:50 + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Remove the organic layer and add it to 150 μL 22 mM pH 2.5 KH_2PO_4/phosphoric acid buffer, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Discard the organic layer, inject a 65 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 Supelco C18
Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH_2PO_4 containing 1.5 mL/L triethylamine, adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 65
Detector: UV 240

CHROMATOGRAM

Retention time: 5.56
Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, nortriptyline
Simultaneous: chlorprothixene, clomipramine, clozapine, flupenthixol, haloperidol, levomepromazine, perphenazine, zuclopenthixol
Noninterfering: benzodiazepines
Interfering: 8-hydroxyclomipramine

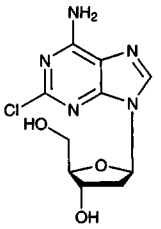
KEY WORDS

serum

REFERENCE

Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites, *J.Chromatogr.B*, **1996**, *675*, 83–88.

Cladribine



Molecular formula: $C_{10}H_{12}ClN_5O_3$
Molecular weight: 285.69
CAS Registry No.: 4291-63-8
Merck Index: 2397

SAMPLE

Matrix: blood
Sample preparation: Add 50 µL 1 µg/mL IS in MeOH:water 20:80 to 1 mL plasma, vortex for 2 s, add 500 µL 1 M sodium carbonate, add 7 mL ethyl acetate, vortex for 90 s, centrifuge at 2000 rpm for 5 min. Freeze in dry ice. Decant the organic layer and evaporate it to dryness under nitrogen, reconstitute with 100 µL MeOH:water 25:75, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: 20 mm long Supelcosil LC-8-DB
Column: 33 × 4.6 3 µm Supelcosil LC-8-DB
Mobile phase: MeOH:5 mM ammonium acetate 25:75
Flow rate: 1
Injection volume: 30
Detector: MS, PE-Sciex API IIIplus triple quadrupole, nebulizer probe 450°, nebulizing gas nitrogen at 80 psi, curtain gas UHP nitrogen at 1.5 L/min, corona discharge needle +3 µA, orifice potential +50 V, collision gas argon at 22 eV, m/z 286

CHROMATOGRAM

Retention time: 1.5
Internal standard: RWJ-29727 (6-amino-2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purine; R.W. Johnson, Raritan NJ) (m/z 304) (2.0)
Limit of quantitation: 0.1 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Moyer,M.D.; Johannsen,T.; Stubbs,R.J. Determination of 2-chlorodeoxyadenosine (cladribine, 2-CdA) in human plasma by liquid chromatography--atmospheric pressure chemical ionization mass spectrometry, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 45–51.

SAMPLE

Matrix: blood
Sample preparation: Add 200 µL ice-cold 400 mM perchloric acid containing 80 mM triethylammonium phosphate to the cell pellet (10^7-5 × 10^8 cells), vortex, add 100 µL ice-cold 1200 mM KOH containing 400 mM ammonium dihydrogen phosphate to reach pH 6.2. Vortex, centrifuge at 14500 g at 4° for 5 min, inject a 90 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: µBondapak C18 Guard-Pak
Column: 250 × 4.6 5 µm Ultrasphere ODS (Beckman Instruments)
Mobile phase: MeOH:80 mM pH 6.1 triethylammonium phosphate buffer 11:89
Flow rate: 1.5
Injection volume: 90
Detector: UV 265

CHROMATOGRAM
Retention time: 29.0

OTHER SUBSTANCES
Extracted: cladribine mono- di-, and triphosphates

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Reichelova,V.; Albertioni,F.; Liliemark,J. Determination of 2-chloro-2'-deoxyadenosine nucleotides in leukemic cells by ion-pair high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 115–123.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 125 pmole IS +5 mL ethyl acetate, vortex for 30 s, centrifuge at 700 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 40 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 mm long 3 μm C18 (Perkin-Elmer)
Mobile phase: MeCN:MeOH:sodium phosphate buffer 5:10:85, pH 3.0
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5
Internal standard: 6-nitroimidazol-6-thioguanine (Guaneran) (5.5)
Limit of detection: 1 nM

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Liliemark,J.; Pettersson,B.; Juliusson,G. Determination of 2-chloro-2'-deoxyadenosine in human plasma, *Biomed.Chromatogr.*, **1991**, *5*, 262–264.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 5 mL acetone + 10 μL Brij 35 solution, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute with 1 mL mobile phase, add IS, inject a 50 μL aliquot.

HPLC VARIABLES
Column: LiChrosorb Si60
Mobile phase: MeOH:water:50 mM KH_2PO_4 5:45:50
Flow rate: 1
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Internal standard: 5,6-dimethylbenzo-1,2,4-triazole-1-β-D-ribofuranoside
Limit of detection: 1 μg/mL

KEY WORDS
serum

REFERENCE
Gajewska,M.; Pawinski,T. Determination of 2-chloro-2'-deoxyadenosine in human blood serum by high-performance liquid chromatography (Abstract 85), *Ther.Drug Monit.*, **1995**, *17*, 404–404.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in water at a concentration of 4 mg/mL, inject a 50-100 μL aliquot. Alternatively, dissolve in water at a concentration of 0.5 mg/mL, inject a 10 μL aliquot (for quantitative impurity profile).

HPLC VARIABLES
Guard column: 5 μm Rainin C18
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: Gradient. A was 100 mM ammonium acetate adjusted to pH 6.55 ± 0.1 with dilute acetic acid. B was MeCN. (a) A:B from 96:4 to 80:20 over 22 min, maintain at 80:20 for 12 min; or (b) (for late-eluting impurities), A:B from 89:11 to 50:50 over 21 min, maintain at 50:50 for 5 min; or (c) (for quantitative impurity profile) A:B from 96:4 to 80:20 over 22 min, maintain at 80:20 for 1 min, to 50:50 over 10 min, maintain at 50:50 for 20 min.
Flow rate: 1.2 ((a), (b)), 1 (c)
Injection volume: 10-100
Detector: UV 264 or MS, Finnigan MAT TSQ-70 triple quadrupole, thermospray, vaporizer 85°, ion source 250°, discharge 2000 V, scan 145-850, scan time 2 s

CHROMATOGRAM
Retention time: 21.5 (A), 7.5 (B)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Weber,J.V.; Sampino,K.; Dunphy,R.; Burinsky,D.J.; Williams,T.; Motto,M.G. Characterization of cladribine and its related componds by high-performance liquid chromatography/mass spectrometry, *J.Pharm.Sci.*, **1994**, *83*, 525–531.

SAMPLE
Matrix: solutions

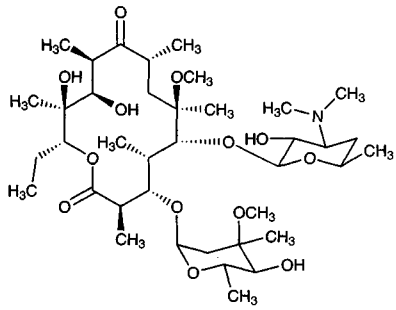
HPLC VARIABLES
Column: 80 × 4.6 3 μm (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 6.8 potassium phosphate buffer 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: fludarabine, analogs, degradation products

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Liquid chromatographic study of acid stability of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 711–714.

Clarithromycin



Molecular formula: $C_{38}H_{69}NO_{13}$
Molecular weight: 747.96
CAS Registry No.: 81103-11-9
Merck Index: 2400

SAMPLE

Matrix: blood
Sample preparation: Mix 200 µL plasma with 50 µL IS, add 200 µL 100 mM sodium carbonate, vortex for 30 s, add 3.5 mL MTBE, mix for 20 min, centrifuge at 2000 g for 10 min. Evaporate the MTBE layer to dryness at 37°, reconstitute the residue in 200 µL mobile phase, mix for 20 min, centrifuge, inject an 80 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 55:45
Flow rate: 1
Injection volume: 80
Detector: E, ESA Coulochem 5100 A, ESA 5010 dual electrode analytical cell at +680 mV and +780 mV, ESA 5020 guard cell +1.0 V

CHROMATOGRAM

Internal standard: azithromycin
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Patel,K.B.; Xuan,D.; Tessier,P.R.; Russomanno,J.H.; Quintiliani,R.; Nightingale,C.H. Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2375–2379.

SAMPLE

Matrix: blood, gastric juice, gastric mucosa, saliva, vitreous humor
Sample preparation: Homogenize 5-20 mg gastric mucosa in 300 µL 10 mM pH 7.4 sodium phosphate buffer with sonication. Add 500 ng roxithromycin in MeOH:water 50:50 to 500 µL plasma, serum, saliva, gastric juice, leucocytes lysate, vitreous humor or 300 µL gastric mucosa homogenate, vortex, add 200 µL 100 mM sodium carbonate and 3 mL MTBE, shake thoroughly (5 × 2 s in an SMI Multi-tube vortexer), centrifuge at 1000 g for 5 min, freeze the aqueous layer in liquid nitrogen or in a freezer at -70° for 15 min. Evaporate the upper organic layer to dryness in a centrifugal vacuum evaporator (Jouan RC 10.22), reconstitute the residue in 250 µL MeOH:water 50:50, inject a 20-50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Zorbax SB CN

Mobile phase: MeCN:MeOH:50 mM Na_2HPO_4 and NaH_2PO_4 buffer 37.5:6.3:56.2, pH 7.5 (The mobile phase was a mixture of 300 mL MeCN, 50 mL MeOH and 450 mL 50 mM Na_2HPO_4 and NaH_2PO_4 buffer.)
Column temperature: 30
Flow rate: 1
Injection volume: 20-50
Detector: E, ESA Coulochem II, guard cell +1.0 V, screening cell E1 +0.50 V, analytical cell E2 +0.80 V

CHROMATOGRAM
Retention time: 16
Internal standard: roxithromycin (18.5)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: azithromycin

KEY WORDS
pharmacokinetics; plasma; serum; leucocytes

REFERENCE
Kees,F.; Spangler,S.; Wellenhofer,M. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 287–293.

SAMPLE
Matrix: gastric juice
Sample preparation: Dilute 500 μL gastric juice with 2.5 mL water, vortex for 1 min, filter (0.45 μm), inject a 50 μL aliquot.

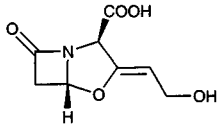
HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 50 mm pH 4.6 phosphate buffer containing 5 mM 1-octanesulfonic acid.)
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 5.8
Limit of detection: 400 ng/mL (water), 780 ng/mL (gastric juice)

OTHER SUBSTANCES
Extracted: degradation products

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Ion-pair high-performance liquid chromatographic assay method for the assessment of clarithromycin stability in aqueous solution and in gastric juice, *J.Chromatogr.B*, **1996**, *682*, 73–78.

Clavulanic acid



Molecular formula: $C_8H_9NO_5$
Molecular weight: 199.16
CAS Registry No.: 58001-44-8, 61177-45-5 (K salt)
Merck Index: 2402
Lednicer No.: 4 180

SAMPLE

Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 195

CHROMATOGRAM

Retention time: 2.71

OTHER SUBSTANCES

Simultaneous: granisetron (UV 300), ticarcillin

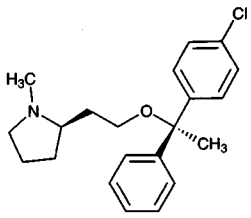
KEY WORDS

stability-indicating; injections; saline

REFERENCE

Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

Clemastine



Molecular formula: $C_{21}H_{26}ClNO$
Molecular weight: 343.90
CAS Registry No.: 15686-51-8, 14976-57-9 (fumarate)
Merck Index: 2405
Lednicer No.: 2 32

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 14.48

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, pen-

thienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 236

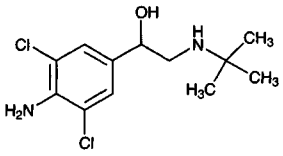
KEY WORDS

chiral; α = 3.04 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Clenbuterol



Molecular formula: $C_{12}H_{18}Cl2N_2O$
Molecular weight: 277.19
CAS Registry No.: 37148-27-9, 21898-19-1(monohydrochloride)
Merck Index: 2407

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 250 mM NaOH + 100 µL 50 ng/mL IS in water + 5 mL diethyl ether:2-butanol 90:10, shake for 10 min, centrifuge at 1500 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL mobile phase, vortex for 2 min, store at 4° overnight, warm to room temperature, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 7 µm LiChrosorb (select B) C18
Column: 125 × 4 7 µm LiChrosorb (select B) C18
Mobile phase: MeCN:buffer 23:77 containing 0.2 mM sodium 1-heptanesulfonate (Buffer was pH 4.0-4.1 phosphate buffer, ionic strength 0.1.)
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, Model 5011 detector, +0.75 V

CHROMATOGRAM

Retention time: 13.5
Internal standard: 4-amino-3,5-dichloro-α-[[(1,1-dimethylpropyl(amino]methyl]benzenemethanol (NAB 760) (10)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: mabuterol

KEY WORDS

horse; plasma

REFERENCE

Qureshi,G.A.; Eriksson,A. Determination of clenbuterol and mabuterol in equine plasma by ion-pair liquid chromatography with electrochemical detection. Chromatographic and electrochemical characteristics, *J.Chromatogr.*, **1988**, *441*, 197–205.

SAMPLE

Matrix: blood, urine
Sample preparation: Vortex 200 µL plasma or urine with 1 mL 1,2-dichloroethane for 10 min and centrifuge for 10 min. Transfer the organic layer to a clean glass tube, evaporate to dryness under nitrogen, redissolve the residue in 200 µL mobile phase, filter, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 Chirex 3022 (chiral stationary phase made of (S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl)ethylamine) (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:ethanol/trifluoroacetic acid 80:10:10 (Ethanol and trifluoroacetic acid were pre-mixed at a ratio of 20:1.)
Column temperature: 23
Flow rate: 0.8
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 9.88 (+), 11.77 (-)
Limit of quantitation: 100 .pico.M

KEY WORDS
plasma; chiral

REFERENCE
Abou-Basha,L.I.; Aboul-Enein,H.Y. Direct enantioselective separation of clenbuterol by chiral HPLC in biological fluids, *Biomed.Chromatogr.*, **1996**, *10*, 69–72.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 10.802

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 13 mM Empore C18 SPE disk (Baker) with 2.5 mL MeOH and 2.5 mL water at 1.5 mL/min. Dissolve tablet in dissolution medium (?). Pass 50 mL through the SPE disk, wash with 2.5 mL water, dry, add 1 mL MeOH and let it soak in for 3 min, elute at 0.5 mL/min, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher cyano

Mobile phase: MeOH:10 mM pH 6.6 phosphate buffer 75:25
Column temperature: 25
Flow rate: 1.3
Injection volume: 50
Detector: UV 214

KEY WORDS
tablets; SPE; comparison with capillary electrophoresis

REFERENCE
Carducci,C.N.; Lucangioli,S.E.; Rodríguez,V.G.; Fernández Otero,G.C. Application of extraction disks in dissolution tests of clenbuterol and levothyroxine tablets by capillary electrophoresis, *J.Chromatogr.A*, **1996**, *730*, 313–319.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 11.8

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: pyrithyldione

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3022 (Phenomenex)

Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 50:35:15 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 248

KEY WORDS
chiral; α = 1.27 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4 5 μm CHIRAL-AGP (ChromTech)
Mobile phase: Isopropanol:15 mM pH 5.0 acetate buffer 1:99
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 3.5, 5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column, *J.Chromatogr.A*, **1995**, *694*, 57–69.

SAMPLE
Matrix: tissue
Sample preparation: Add 5 mL 1 M HCl to the tissue, vortex for 1 min, sonicate for 10 min, and centrifuge at 2100 g for 20 min. Remove the supernatant and add 5 mL 1 M EDTA in 4 M NaOH. Adjust the pH to 12.2 ± 0.1 with 10 M NaOH, keep at room temperature for 2 h. Add 5 mL diethyl ether to the sample, vortex for 1 min, shake for 4 min, and centrifuge at 2100 rpm for 2 min. Remove the diethyl ether layer and dry it under nitrogen at 80°. Add another 5 mL of diethyl ether to the aqueous phase, vortex, centrifuge, and add the ether layer to the same vial that contains the residue from the previous extraction. Dry under nitrogen at 80°, dissolve the residue in 200 μL of 1% formic acid, vortex for 5 min, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18e
Column: 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: MeOH:water:formic acid 34:66:1
Column temperature: 40
Flow rate: 1.0
Injection volume: 50
Detector: E, Hewlett Packard Model 1049A, glassy carbon electrode 1.3V for 999 ms pulsed to -2.0V for 200 ms (to prevent fouling electrode), solid state Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8

Limit of detection: 5 ng/mL

KEY WORDS
retina; cow

REFERENCE
Lin,L.A.; Tomlinson,J.A.; Satzger,R.D. Detection of clenbuterol in bovine retinal tissue by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *762*, 275–280.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg WCX-SPE weak cation-exchange SPE cartridge (Baker) with 10 mL EtOH, 3 mL water, 3 mL 100 mM pH 6 sodium dihydrogen phosphate, and 3 mL water. Wash a RidaScreen immunoaffinity (IA) cartridge (Bioman Products, Canada) with 2 mL pH 7.4 phosphate buffered saline (PBS). Add 30 mL 10 mM HCl to 6 g tissue, shake for 20 s, sonicate for 15 min. Shake and heat at 80° for 30 min. Cool at -15°, centrifuge at 9200 g at 5° for 20 min. Adjust the pH of the supernatant to 6 with 1 M NaOH. Add to the SPE cartridge, wash with 4 mL water and 4 mL EtOH. Elute with 5 mL 2% ammonium hydroxide in EtOH. Evaporate eluate to 100 μL under a stream of nitrogen at 30°. Dilute to 1 mL with PBS. Add 1 mL to the IA cartridge, wash with 500 μL PBS, wash with 1 mL EtOH:water 20:80. Elute with 2 mL EtOH:water 80:20. Evaporate the eluate to 100 μL under a stream of nitrogen at 30°, dilute to 400 μL with mobile phase B, mix. Inject a 150 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeOH. B was water containing 10 mM acetic-acid ammonium acetate buffer adjusted to pH 4.6 with acetic acid. A:B from 30:70 to 70:30 over 5 min
Flow rate: 0.8
Injection volume: 150
Detector: UV 245

CHROMATOGRAM
Retention time: 4.6
Limit of detection: 300 pg/g

KEY WORDS
cow; liver; muscle; SPE; immunoaffinity

REFERENCE
Lawrence,J.F.; Ménard,C. Determination of clenbuterol in beef liver and muscle tissue using immunoaffinity chromatographic cleanup and liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *696*, 291–297.

SAMPLE
Matrix: urine
Sample preparation: Add ammonia to 20 mL urine to adjust the pH to ca. 11. Add the sample to a Chem Elut SPE cartridge until completely absorbed. After 10 min elute with three 20 mL portions of n-hexane, evaporate the eluate to dryness. Redissolve in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Nova-Pak C18
Column: 150 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeOH:3.5mM pH 3.0 $(NH_4)_2HPO_4$ 90:10
Flow rate: 1.5

Detector: UV 246

CHROMATOGRAM

Retention time: 3.9
Limit of detection: 1 ng/mL

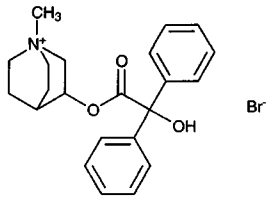
KEY WORDS

comparison with GC-MS/EI/SIM and ELISA; cow; SPE

REFERENCE

Ramos,F.; Castilho,M.C.; Noronha Da Silveira,M.I. Occurrence of β2-adrenergic agonist residues in urine of animal meat producers in Portugal, *J.AOAC Int.*, **1998**, *81*, 544–548.

Clidinium bromide



Molecular formula: $C_{22}H_{26}BrNO_3$
Molecular weight: 432.36
CAS Registry No.: 3485-62-9
Merck Index: 2412

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.27

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Open capsules, weigh out amount equivalent to 5 mg clidinium bromide, add 15 mL water, sonicate for 10 min with gentle swirling. Make up to 25 mL with water, centrifuge, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 µm Partisil 10 ODS-3
Mobile phase: MeCN:300 mM $(NH_4)H_2PO_4$ 32:68, adjust pH to 4.3 ± 0.1 with 10% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 235

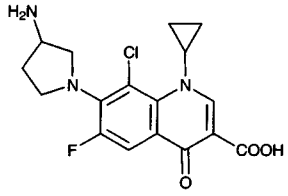
CHROMATOGRAM
Retention time: 7.1

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, impurities

KEY WORDS
capsules

REFERENCE
Yuen,S.M.; Lehr,G. Liquid chromatographic determination of clidinium bromide and clidinium bromide-chlordiazepoxide hydrochloride combinations in capsules, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 461–464.

Clinafloxacin



Molecular formula: $C_{17}H_{17}ClFN_3O_3$
Molecular weight: 365.79
CAS Registry No.: 105956-97-6, 105956-99-8 (HCl)
Merck Index: 2413

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 25 μL water + 25 μL 15 μg/mL IS + 50 μL MeCN: perchloric acid 80:20, centrifuge at 10687 g for 10 min, inject a 150 μL supernatant aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm BDS-Hypersil C18
Mobile phase: MeCN:buffer 20:80, (Buffer (ion pairing solution) was 50 mM citric acid containing 1.15 mM tetrabutylammonium hydroxide and 0.1% ammonium perchlorate, adjusted to pH 4.0.)
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM

Retention time: 6
Internal standard: 1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-[3-[(methylamino)methyl]-1-pyrrolidinyl]-4-oxo-3-quinolinecarboxylic acid (PD 118012) (7.9)
Limit of quantitation: 25 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Brodfuehrer,J.I.; Priebe,S.; Guttendorf,R. Achiral and chiral high-performance liquid chromatographic methods for clinafloxacin, a fluoroquinolone antibacterial, in human plasma, *J.Chromatogr.B*, **1998**, *709*, 265–272.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge with two 1 mL portions of MeCN, four 1 mL portions of MeCN:water 50:50, and two 1 mL portions of water. 1 mL Plasma + 100 μL water + 50 μL 20 μg/mL IS + 1 mL 0.25 mM decylamine in water, mix, add to the SPE cartridge, wash with 1 mL MeCN:water 5:95, elute with 1 mL MeOH:water 40:60, evaporate under nitrogen at 45°, reconstitute the residue in 200 μL MeOH:water 15:85, inject a 125 μL aliquot. (Protect from light!)

HPLC VARIABLES

Column: 150 × 4 5 μm Daicel Crownpak CR(+) (Chiral Technologies, Exton, PA)
Mobile phase: MeOH:water 12:88 containing 0.1 mM decylamine, adjusted to pH 2.0 with perchloric acid
Column temperature: 35
Flow rate: 1
Injection volume: 125
Detector: UV 340

CHROMATOGRAM

Retention time: 31.3 ± 1.0 (R), 36.9 ± 1.0 (S)

Internal standard: R-7-[3-(1-amino-1-methyl-ethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, monohydrochloride (PD 138312) (54.7 ± 1.0)

Limit of quantitation: 40 ng/mL

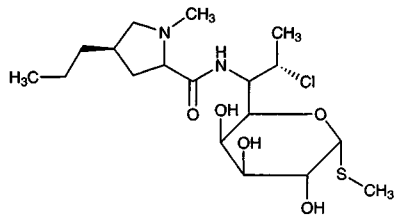
KEY WORDS

plasma; chiral; SPE; pharmacokinetics; protect from light

REFERENCE

Brodfuehrer,J.I.; Priebe,S.; Guttendorf,R. Achiral and chiral high-performance liquid chromatographic methods for clinafloxacin, a fluoroquinolone antibacterial, in human plasma, *J.Chromatogr.B*, **1998**, *709*, 265–272.

Clindamycin



Molecular formula: $C_{18}H_{33}ClN_2O_5S$

Molecular weight: 424.99

CAS Registry No.: 18323-44-9, 58207-19-5 (HCl monohydrate), 36688-78-5 (palmitate), 25507-04-4 (palmitate HCl), 24729-96-2 (phosphate)

Merck Index: 2414

SAMPLE

Matrix: blood

Sample preparation: Prepare a silica SPE cartridge. Fill a 3 mL cartridge with 500 mg Silica gel 60 (Merck). Condition it with 2.5 mL MeOH and with 2.5 mL water. Spike 500 μL plasma or serum with 30 μL 6 ng/μL propranolol, add to the SPE cartridge. Wash with 1 mL water, elute with 3 mL MeOH (added dropwise). Evaporate eluates to dryness under a gentle stream of nitrogen. Reconstitute the residue in 200 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Keystone ODS2

Mobile phase: MeCN:THF:50 mM pH 5.00 phosphate buffer 24:1:75

Flow rate: 1

Injection volume: 30

Detector: UV 204

CHROMATOGRAM

Retention time: 12

Internal standard: propranolol (21)

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; serum; SPE; pharmacokinetics

REFERENCE

Liu,C.-M.; Chen,Y.-K.; Yang,T.-H.; Hsieh,S.-Y.; Hung,M.-H.; Lin,E.T. High-performance liquid chromatographic determination of clindamycin in human plasma or serum: application to the bioequivalency study of clindamycin phosphate injections, *J.Chromatogr.B*, **1997**, *696*, 298–302.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.962

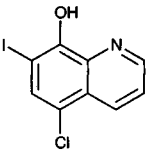
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clioquinol



Molecular formula: C_9H_5ClINO
Molecular weight: 305.50
CAS Registry No.: 130-26-7
Merck Index: 5052

SAMPLE

Matrix: formulations
Sample preparation: 1 g Ointment + 30 mL trimethylpentane, warm on a water bath until ointment melts, add 10 mL 4% bromobenzene in MeOH:water 80:20, extract with 30 mL methanol:50 mM phosphoric acid 80:20 then twice with 20 mL methanol:50 mM phosphoric acid 80:20, combine extracts, cool, make up to 100 mL with methanol:50 mM phosphoric acid 80:20, inject 20 μL aliquot.

HPLC VARIABLES

Column: 225 × 4 Hypersil-ODS
Mobile phase: MeOH:50 mM phosphoric acid 80:20
Flow rate: 2
Injection volume: 20
Detector: UV 256

CHROMATOGRAM

Retention time: 5
Internal standard: bromobenzene (3.5)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: hydrocortisone

KEY WORDS

ointment

REFERENCE

Phoon,K.W.; Stubley,C. Rapid method for the simultaneous analysis of hydrocortisone and clioquinol in topical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *246*, 297–303.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out ointment or cream containing 30 mg clioquinol, add 50 mL THF, warm on steam bath to dissolve, make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL 100 mg/mL nickel(II) chloride hexahydrate in MeOH, add 5 mL 400 μg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm phenyl (Waters)
Mobile phase: MeCN:MeOH:water 27:18:55 containing 1 mM nickel chloride and 10 mM ammonium acetate
Flow rate: 1.2-1.5
Injection volume: 10-20
Detector: UV 273

CHROMATOGRAM

Retention time: 7.5

Internal standard: diphenylamine (11.5)

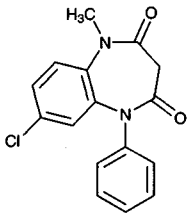
KEY WORDS

ointment; cream

REFERENCE

Wojtowicz,E.J. Reverse-phase liquid chromatographic determination of clioquinol in cream and ointment preparations: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 562–563.

Clobazam



Molecular formula: $C_{16}H_{13}ClN_2O_2$
Molecular weight: 300.74
CAS Registry No.: 22316-47-8
Merck Index: 2417
Lednicer No.: 2 406

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 4.00

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 229.9

CHROMATOGRAM

Retention time: 19.19

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4.6 10 μm LiChrosorb RP8

Mobile phase: MeOH:100 mM pH 7.0 tetraethylammonium phosphate buffer 39:48
Flow rate: 2
Injection volume: 75
Detector: UV 230 following post-column reaction. The column effluent flowed through a 20 m × 0.5 mm ID knitted PTFE coil irradiated by a 15 w low-pressure mercury lamp (Original Hanau TNN 15/32) to the detector., F ex 364 em 400 following post-column reaction. The column effluent flowed through a 20 m × 0.5 mm ID knitted PTFE coil irradiated by a 15 w low-pressure mercury lamp (Original Hanau TNN 15/32) to the detector.

CHROMATOGRAM

Retention time: 5
Limit of detection: 1 ng

OTHER SUBSTANCES

Extracted: metabolites

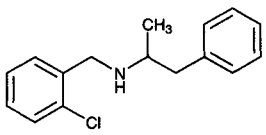
KEY WORDS

post-column reaction; post-column photochemical derivatization

REFERENCE

Uihlein,M.; Schwab,E. A novel reactor for photochemical post-column derivatization in HPLC, *Chromatographia*, **1982**, *15*, 140–146.

Clobenzorex



Molecular formula: $C_{16}H_{18}ClN$
Molecular weight: 259.78
CAS Registry No.: 13364-32-4, 5843-53-8 (HCl)
Merck Index: 2421

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.912

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 2 mg of the hydrochloride in 30 mL 450 mM NaOH, extract with 30 mL chloroform. Add 10 µL phenylisothiocyanate to the chloroform solution, evaporate to dryness under a stream of air, reconstitute with 1 mL MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 µBondapak C18
Mobile phase: MeOH:water:acetic acid 65:34:1
Flow rate: 1.5
Injection volume: 5

Next Page

Detector: UV 254

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Simultaneous: 3- and 4-chloro analogs

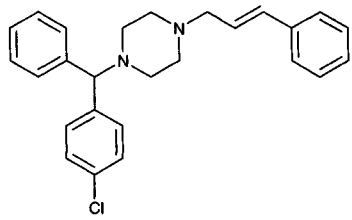
KEY WORDS

derivatization

REFERENCE

Noggle,F.T.,Jr.; Clark,C.R.; Andurkar,S.V.; DeRuiter,J. Liquid chromatographic analysis of regioisomers and enantiomers of N-(chlorobenzyl)-α-methylphenethylamines: Analogues of clobenzorex, *J.Liq.Chromatogr.*, **1990**, *13*, 763–777.

Clocinizine



Molecular formula: $C_{26}H_{27}ClN_2$
Molecular weight: 402.97
CAS Registry No.: 298-55-5
Merck Index: 2428

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 20.432

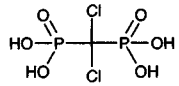
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clodronic acid



Molecular formula: $CH_4Cl_2O_6P_2$
Molecular weight: 244.89
CAS Registry No.: 10596-23-3
Merck Index: 2432

SAMPLE

Matrix: blood, solutions, tissue
Sample preparation: Serum. Add 250 μL human serum to 250 μL MeOH, mix, centrifuge. Evaporate 300 μL aliquot of the supernatant to dryness under a stream of nitrogen. Reconstitute the residue in 400 μL water. Inject a 20 μL aliquot. Tissue. Add 400 μL MeOH to 400 μL liver homogenate. Mix, centrifuge, evaporate a 450 μL aliquot of the supernatant to dryness under a stream of nitrogen at 20°. Reconstitute the residue with 600 μL water. Inject a 20 μL aliquot. Solutions. Prepare solutions in 50 mM pH 7.4 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Kromasil 100 RP-C8 (Higgins Analytical, USA)
Mobile phase: MeOH:buffer 3:97 (Buffer was 100 mM ammonium acetate containing 230 mM butylamine, adjusted to pH 4.6 with acetic acid.)
Flow rate: 1.2
Injection volume: 20
Detector: ELSD, Sedex 55 (Sedere, France), 70°, nebulizing gas (dried and filtered air) pressure 2.2 bar

CHROMATOGRAM

Retention time: 4.26
Limit of detection: 37.5 μg/mL
Limit of quantitation: 50 μg/mL

KEY WORDS

serum; liver; rabbit; human

REFERENCE

Niemi,R.; Taipale,H.; Ahlmark,M.; Vepsäläinen,J.; Järvinen,T. Simultaneous determination of clodronate and its partial ester derivatives by ion-pair reversed-phase high-performance liquid chromatography coupled with evaporative light-scattering detection, *J.Chromatogr.B*, **1997**, *701*, 97–102.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with water to a concentration of 850 μg/mL clodronic acid. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: AG5 (Dionex)
Column: 250 × 4 15 μm IonPac AS5 (Dionex)
Mobile phase: Gradient. 20 mM NaOH to 100 mM NaOH over 20 min
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: Conductivity, Dionex ED40, thermostated DS3 cell in conductivity mode, detection carried out using an Anion Self Regenerating Suppressor (ASRS-1) in recycle mode

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES
Simultaneous: carbonylbisphosphonic acid, chloride, diprotic acid, methylenebisphosphonic acid, monochloromethylenebisphosphonic acid, nitrate, phosphate, sulfate, triprotic acid

KEY WORDS
injections

REFERENCE
Taylor,G.E. Determination of impurities in clodronic acid by anion-exchange chromatography, *J.Chromatogr.A*, **1997**, *770*, 261–271.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water to a concentration of 400 μg/mL, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 6 μm IC-Pak HR anion-exchange (Waters)
Mobile phase: 12 mM nitric acid
Flow rate: 1
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 6
Limit of detection: 1000 ng/mL

KEY WORDS
injections; indirect UV detection; rugged

REFERENCE
Tsai,E.W.; Chamberlin,S.D.; Forsyth,R.J.; Bell,C.; Ip,D.P.; Brooks,M.A. Determination of bisphosphonate drugs in pharmaceutical dosage formulations by ion chromatography with indirect UV determination, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 983–991.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 30.4

OTHER SUBSTANCES
Simultaneous: alendronate, etidronate, neridronate, olpadronate, pamidronate

KEY WORDS
derivatization; complexation

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1545–1550.

SAMPLE
Matrix: urine
Sample preparation: Inject directly.

HPLC VARIABLES
Guard column: 50 × 4 10 μm HPLC AG7 anion-exchange (Dionex)
Column: 250 × 4 10 μm HPIC AS7 anion-exchange (Dionex)
Mobile phase: 30 mM nitric acid adjusted to pH 2.4 with dilute NaOH
Flow rate: 1
Injection volume: 100
Detector: UV 550 following post-column reaction. Column effluent with reagent pumped at 1 mL/min, allow to flow through 1.2 m × 0.25 mm i.d. PEEK tubing to the detector. (Reagent was 4.5 mL 0.2 mM thorium solution + 100 mL 0.4 mM disodium EDTA in water, mix, add 60 mL buffer, add 1.9 mL 500 μg/mL xylenol orange in water, shake vigorously, adjust pH so that the combined pH of the reagent and the column effluent will be 6.0-6.3, dilute to 500 mL with water, allow to stand for at least 1 h before use. thorium solution was 1.104 g $Th(NO_3)_3.4H_2O$ in 20 mL 2 M nitric acid, dilute to 500 mL with water to give a 4 mM solution, dilute an aliquot further to give a 0.2 mM solution. Buffer was 10.1 mL ethylenediamine in ice-cold water, add 15 mL concentrated HCl slowly with stirring so as to keep the temperature below 20°, adjust pH to 7.3 with dilute HCl, dilute to 250 mL with water.)

CHROMATOGRAM
Retention time: 12
Limit of detection: 1100 ng/mL

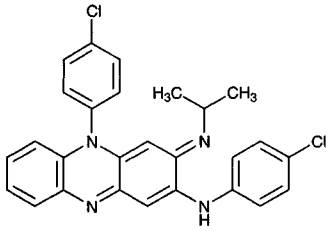
OTHER SUBSTANCES
Extracted: clodronate esters

KEY WORDS
post-column reaction

REFERENCE
Virtanen,V.; Lajunen,L.H. High-performance liquid chromatographic method for simultaneous determination of clodronate and some clodronate esters, *J.Chromatogr.*, **1993**, *617*, 291–298.

Clofazimine

Molecular formula: $C_{27}H_{22}Cl_2N_4$
Molecular weight: 473.40
CAS Registry No.: 2030-63-9
Merck Index: 2433



SAMPLE

Matrix: blood

Sample preparation: Condition a Prep Sep CN SPE cartridge (Fisher) with 3 mL MeOH, 5 mL water, and 5 mL 100 mM pH 6.0 phosphate buffer, do not allow to go dry. 1 mL Plasma + 100 µL MeOH:water 10:90 + 5 mL 100 mM pH 6.0 phosphate buffer, vortex, let stand, add to the SPE cartridge, air dry for 2 min, add 50 µL 3.4 µg/mL salicylic acid, elute with four 1 mL portions of THF:MeCN:MeOH 40:40:20 containing 0.7 mM hexanesulfonic acid. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 3.9 µBondapak C18
Mobile phase: MeOH:THF:0.5% acetic acid 5:40:55 containing 2.5 mM hexanesulfonic acid
Flow rate: 1.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 6
Internal standard: salicylic acid (3.3)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Simultaneous: dapsone

KEY WORDS

plasma; SPE; rabbit; pharmacokinetics; human

REFERENCE

Krishnan,T.R.; Abraham,I. A rapid and sensitive high performance liquid chromatographic analysis of clofazimine in plasma, *Int.J.Lepr.Other Mycobact.Dis.*, **1992**, *60*, 549–555.

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Add 2 µg IS in dichloromethane to a glass tube. Evaporate by heating to 40°. Homogenize 100 mg tissue in 1 mL water. Pour the homogenate into the tube coated with IS, add 1 mL 5 M NaOH, add 2 mL dichloromethane, mix on a blood tube mixer for 20 min, sonicate for 5 min, centrifuge at 1800 g for 15 min. Discard the aqueous layer, add 1.5 mL 10% NaOH in EtOH to the organic layer, heat at 80° until the bubbling stopped (indicating complete evaporation of the dichloromethane), add 6 mL cold water, add 2 mL dichloromethane, mix for 5 min on a blood tube mixer, centrifuge at 1400 g for 10 min, discard the aqueous layer, wash the organic layer twice more in the same fashion. Remove a 1.1 mL aliquot of the organic layer and evaporate it to dryness by heating at 40° for 2 h. Reconstitute with 120 µL 0.6% (v/v) acetic acid in THF, inject a 20 µL aliquot. Serum. Add 2 µg IS in dichloromethane to a glass tube. Evaporate by heating to 40°. Add 1 mL serum, 1 mL 5 M NaOH, and 2 mL dichloromethane, mix on a

blood tube mixer for 20 min, sonicate for 5 min, centrifuge at 1800 g for 15 min. Remove a 1.1 mL aliquot of the organic layer and evaporate it to dryness by heating at 40° for 2 h. Reconstitute with 60 μL 0.6% (v/v) acetic acid in THF, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 300 × 3.9 10 μm Bondclone C18 (Phenomenex)
Mobile phase: THF:water:acetic acid 40:59.4:0.6 containing 471 mg/L hexanesulfonic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Retention time: 5.6
Internal standard: B4090 (4.4)
Limit of quantitation: 10 ng/mL (serum, tissue) 20 ng/g (fat)

KEY WORDS

fat; serum; rat

REFERENCE

O'Connor,R.; O'Sullivan,J.F.; O'Kennedy,R. Determination of serum and tissue levels of phenazines including clofazimine, *J.Chromatogr.B*, **1996**, *681*, 307–315.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 NewGuard RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 38:62 (Buffer was 50 mM KH_2PO_4:triethylamine 61.5:0.5 adjusted to pH 4.2 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM

Retention time: 90

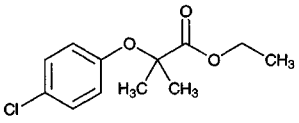
OTHER SUBSTANCES

Simultaneous: medazepam, rifabutin
Noninterfering: amikacin, isoniazid, streptomycin, zidovudine

REFERENCE

Lewis,R.C.; Hatfield,N.Z.; Narang,P.K. A sensitive method for quantitation of rifabutin and its desacetyl metabolite in human biological fluids by high-performance liquid chromatography (HPLC), *Pharm.Res.*, **1991**, *8*, 1434–1440.

Clofibrate



Molecular formula: $C_{12}H_{15}ClO_3$
Molecular weight: 242.7
CAS Registry No.: 637-07-0
Merck Index: 2436
Lednicer No.: 1 119

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.267

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: tissue
Sample preparation: Powder rat liver under liquid nitrogen. Homogenize tissue with cooled 6% perchloric acid, centrifuge at 8000 g for 3 min, inject a 20-50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18
Column: 250 × 4.6 5 µm Supelcosil LC-18
Mobile phase: MeOH:28 mM pH 4.2 phosphate buffer 52:48
Flow rate: 1.5
Injection volume: 20-50

Detector: UV 230

CHROMATOGRAM
Retention time: 4.7
Limit of detection: 2 pmole

OTHER SUBSTANCES
Extracted: clofibroyl coenzyme A

KEY WORDS
rat; liver

REFERENCE
Lygre,T.; Aarsaether,N.; Stensland,E.; Aarsland,A.; Berge,R.K. Separation and measurement of clofibroyl coenzyme A and clofibric acid in rat liver after clofibrate adminstration by reversed-phase high-performance liquid chromatography with photodiode array detection, *J.Chromatogr.*, **1986**, *381*, 95–105.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 20-fold with 100 mM pH 2.0 phosphate buffer, extract twice with two volumes of ethyl acetate, centrifuge at 5000 g for 5 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen below 30°. Reconstitute in 0.2-1 mL mobile phase, inject an aliquot.

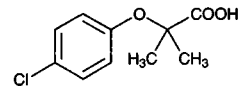
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 75:25 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 224

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: glucuronides

REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

Clofibric acid



Molecular formula: $C_{10}H_{11}ClO_3$
Molecular weight: 214.65
CAS Registry No.: 882-09-7
Merck Index: 2437

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg C2 SPE cartridge (Analytichem) with 2 mL MeOH and 1 mL water. 100 μL Plasma + 20 μL 200 μg/mL ketoprofen in MeOH + 500 μL 1 M HCl, vortex for 15 s, add to the SPE cartridge, rinse out tube with 1 mL water, add rinse to the SPE cartridge, elute with 1 mL mobile phase, vortex the eluate, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 30-40 μm pellicular Vydac Reversed-Phase
Column: 75 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeOH:buffer 42:58 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 7.0 with 50% aqueous NaOH.)
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems LC-4B, LC-17 thin-layer glassy carbon working electrode +1.10 V, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through an air-cooled 7.9 m × 0.3 mm ID PTFE coil irradiated by an SC3-9 ultraviolet lamp (UVP, Inc.) to the detector.

CHROMATOGRAM

Retention time: 2
Internal standard: ketoprofen (2.7)
Limit of detection: 200 ng/mL

KEY WORDS

post-column reaction; plasma; SPE; post-column photochemical derivatization

REFERENCE

Bachman,W.J.; Stewart,J.T. HPLC-photolysis-electrochemical detection in pharmaceutical analysis: Application to the determination of clofibric acid in human plasma, *J.Liq.Chromatogr.*, **1989**, *12*, 2947–2959.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 1 μg/mL propyl paraben in MeCN (if clofibric acid concentration is 15-60 μg/mL add 25 μL MeCN), shake gently, vortex for 30 s, centrifuge at 5000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: Adsorbosil C18 (Alltech)
Column: 150 × 4.6 5 μm Microsorb-MV
Mobile phase: MeCN:water:acetic acid 45:55:1
Flow rate: 0.8
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 7.12

Internal standard: propyl paraben (5.56)
Limit of quantitation: 1.5 μg/mL

OTHER SUBSTANCES
Simultaneous: bezafibrate, clofibrate, fenofibrate, fenofibric acid, gemfibrozil

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Lau-Cam,C.; Theofanopoulos,V.; Spireas,S.S. Simplified HPLC method with spectrophotometric detection for the assay of clofibric acid in rat plasma, *J.Liq.Chromatogr.*, **1995**, *18*, 3945–3954.

SAMPLE
Matrix: blood, urine
Sample preparation: 200 μL Plasma or 100 μL urine + 500 (plasma) or 300 (urine) mg NaCl + 1 (plasma) or 0.3 (urine) mL pH 4 buffer + 5 mL n-hexane:EtOH 90:10, shake horizontally for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, add 50 μL toluene and evaporate it to remove traces of water. Reconstitute the residue in 500 μL dichloromethane, add 50 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 50 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 50 μL 1 mg/mL FLOPA, vortex, let stand at room temperature for 2 h, evaporate to dryness, reconstitute in 500 μL mobile phase, inject a 50 μL aliquot. (FLOPA is the corresponding amine hydrochloride from (+)-(S)-flunoxaprofen. Synthesis is as follows (protect from light). 500 mg (+)-(S)-Flunoxaprofen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, add 600 mg sodium azide dissolved in ice water with stirring, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give crystals (mp 91°). Dissolve in ether, add 0.5 M HCl in ether, filter, dissolve solid in a small volume of MeOH, precipitate with ether, dry FLOPA over phosphorus pentoxide under vacuum (Pharm.Res. 1990, 7, 1262).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Gradient. A was n-hexane:chloroform:EtOH 100:10:0.75. B was n-hexane:chloroform:EtOH 100:10:20. A:B 100:0 for 10 min, 50:50 for 5 min, 100:0 for 5 min (stepwise).
Flow rate: 2
Injection volume: 50
Detector: F ex 305 em 355

CHROMATOGRAM
Retention time: 8
Internal standard: clofibric acid

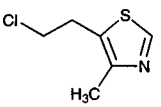
OTHER SUBSTANCES
Extracted: beclobrate

KEY WORDS
clofibric is IS; derivatization; normal phase; plasma

REFERENCE

Mayer,S.; Mutschler,E.; Spahn-Langguth,H. Pharmacokinetic studies with the lipid-regulating agent beclobrate: enantiospecific assay for beclobric acid using a new fluorescent chiral coupling component (S-FLOPA), *Chirality*, **1991**, *3*, 35–42.

Clomethiazole



Molecular formula: C_6H_8ClNS
Molecular weight: 161.65
CAS Registry No.: 533-45-9, 6001-74-7 (HCl), 1867-58-9 (ethanedisulfonate)
Merck Index: 2444

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 249.9

CHROMATOGRAM

Retention time: 15.958

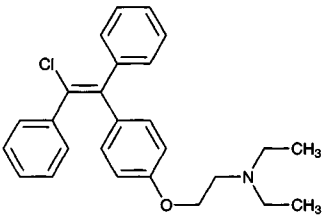
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clomiphene



Molecular formula: $C_{26}H_{28}ClNO$
Molecular weight: 405.97
CAS Registry No.: 911-45-5, 50-41-9 (citrate)
Merck Index: 2446
Lednicer No.: 1 105

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 1 mL pH 9 borate buffer, vortex, add 9 mL redistilled ether, vortex for 2 min, centrifuge at 1600 g for 15 min. Remove the organic layer and dry it over 1 g anhydrous sodium sulfate, centrifuge at 1600 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 75 μL mobile phase, vortex for 2 min, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 6 μm Zorbax Sil
Mobile phase: Chloroform:MeOH 80:20
Flow rate: 0.8
Injection volume: 10
Detector: F following post-column reaction. The column effluent flowed through a 3 m × 0.3 mm ID PTFE coil irradiated by a medium-pressure mercury lamp (Hanovia) with water cooling to the detector.

CHROMATOGRAM

Retention time: 7 (cis-clomiphene), 7.5 (trans-clomiphene)
Limit of detection: 350 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

post-column reaction; post-column photochemical derivatization; plasma; normal phase

REFERENCE

Harman,P.J.; Blackman,G.L.; Phillipou,G. High-performance liquid chromatographic determination of clomiphene using post-column on-line photolysis and fluorescence detection, *J.Chromatogr.*, **1981**, *225*, 131–138.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 2 mL diethyl ether, extract, centrifuge at 2000 rpm at 4° for 10 min, repeat extraction. Combine the organic phases and evaporate them to dryness under a stream of nitrogen at 37°. Reconstitute the residue in 250 μL MeOH, centrifuge at 2000 rpm at 4° for 10 min, inject a 10-100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:10 mM KH_2PO_4:300 mM phosphoric acid :water 42:20:10:28
Flow rate: 2.8
Injection volume: 10-100
Detector: F ex 258 em 378 following post-column reaction. The column effluent flowed through a 6.5 m × 0.35 mm × 1.5 mm o.d. PTFE tube irradiated with a Philips HPK 125 watt high pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 12
Internal standard: clomiphene (9)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: tamoxifen
Simultaneous: metabolites

KEY WORDS
plasma; clomiphene is IS; post-column reaction; post-column photochemical derivatization

REFERENCE
Milano,G.; Etienne,M.C.; Frenay,M.; Khater,R.; Formento,J.L.; Renee,N.; Moll,J.L.; Francoual,M.; Berto,M.; Namer,M. Optimised analysis of tamoxifen and its main metabolites in the plasma and cytosol of mammary tumours, *Br.J.Cancer*, **1987**, *55*, 509–512.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma + 500 ng IS in MeOH, mix, add 500 μL 3 M NaCl, add 1 mL water, mix, add to SPE cartridge, wash with 3 mL water, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:MeOH:water:1% ammonium chloride:1% potassium carbonate 950: 30:20:4:8
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: F ex 247 em 378 following post-column photochemical derivatization in a 15 m × 0.3 mm PTFE tube knitted on a mercury lamp (UV 254)

CHROMATOGRAM
Retention time: 6.8 (E isomer), 7.2 (Z isomer)
Internal standard: 1-(4-diethylaminoethoxy)phenyl-1,2-diphenylethanol (4.7)
Limit of detection: 0.4 ng/mL
Limit of quantitation: 1.25 ng/mL (E), 0.75 ng/mL (Z)

KEY WORDS
plasma; SPE; post-column photochemical derivatization

REFERENCE
Ürmös,I.; Benkö,S.M.; Klebovich,I. Simple and rapid determination of clomiphene *cis* and *trans* isomers in human plasma by high-performance liquid chromatography using on-line post-column photochemical derivatization and fluorescence detection, *J.Chromatogr.*, **1993**, *617*, 168–172.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Photolyze clomiphene in chloroform, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 33 (sic) 10 μm μPorasil
Mobile phase: MeCN:dichloromethane 20:80 containing 0.1% methenamine
Flow rate: 1.6

Injection volume: 20
Detector: UV 298

CHROMATOGRAM

Retention time: 9 (E isomer), 11 (Z isomer)

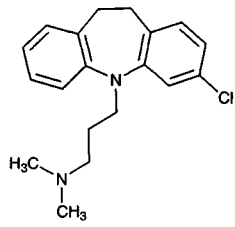
KEY WORDS

normal phase

REFERENCE

Frith,R.G.; Phillipou,G. Application of clomiphene photolysis to assays based on analysis of the derived phenanthrenes, *J.Chromatogr.*, **1986**, *367*, 260–266.

Clomipramine



Molecular formula: $C_{19}H_{23}ClN_2$
Molecular weight: 314.86
CAS Registry No.: 303-49-1, 17231-77-6 (HCl)
Merck Index: 2447

SAMPLE

Matrix: blood
Sample preparation: Add 500 μL 40 ng/mL IS in water to 500 μL plasma. Add to an activated 100 mg Bond-Elut C8 SPE cartridge. Wash with 1 mL water, 1.5 mL MeCN: water 50:50, and 500 μL MeCN. Elute with 1 mL MeOH, dry the eluate under a stream of nitrogen. Reconstitute the residue in 50 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Injection volume: 20
Detector: E, Coulochem 5100A, 5011 analytical cell +400 mV screen electrode, 700 mV sample electrode

CHROMATOGRAM

Internal standard: imipramine
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics; SPE

REFERENCE

Müller,F.O.; Schall,R.; Mogilnicka,E.M.; Groenewoud,G.; Hundt,H.K.L.; Luus,H.G.; Middle,M.V.; Swart,K.J.; De Vaal,A.C. Relative bioavailability of four clomipramine hydrochloride tablet products, *Biopharm.Drug Dispos.*, **1996**, *17*, 81–90.

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM

Retention time: 18.87
Internal standard: clovoxamine (6.504)
Limit of quantitation: 7 ng/mL(UV 226, UV 400); 10 ng/mL(UV 254)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, desipramine, fluoxetine, imipramine, maprotiline, nortriptyline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 5 M NaOH, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it under a gentle stream of nitrogen at 20 °, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RP C18 (Brownlee)
Column: 150 × 4.6 5 μm Microsorb MV
Mobile phase: MeCN:water 55:45 containing 10 mM pH triethylamine, adjusted to pH 4.8 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Retention time: 7.4
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: fluoxetine, norfluoxetine

KEY WORDS
mouse; serum; clomipramine is IS

REFERENCE
Holladay,J.W.; Dewey,M.J; Yoo,S.D. Quantification of fluoxetine and norfluoxetine serum levels by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1997**, *704*, 259–263.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase (J.Liq.Chromatogr. 1981, 4, 849).

HPLC VARIABLES
Guard column: 23 × 3.9 Bondapak/Corasil C 18

Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 8.5
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: maprotiline
Simultaneous: amoxapine, amitriptyline, chlordiazepoxide, chlorpromazine, cimetidine, desipramine, diazepam, doxepin, flurazepam, imipramine, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine
Interfering: nortriptyline

KEY WORDS
clomipramine is IS; plasma

REFERENCE
Wong,S.H.Y.; Waugh,S.W. Determination of the antidepressants maprotiline and amoxapine, and their metabolites, in plasma by liquid chromatography, *Clin.Chem.*, **1983**, *29*, 314–318.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 4
Internal standard: desipramine hydrochloride (12)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: desmethylclomipramine, maprotiline, protriptyline, metabolites
Also analyzed: doxepin, desmethyldoxepin, amitriptyline, nortriptyline, imipramine, 2-hydroxyimipramine, 2-hydroxydesipramine
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

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KEY WORDS
plasma

REFERENCE
Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE
Matrix: blood
Sample preparation: Take 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: clomipramine

OTHER SUBSTANCES
Simultaneous: imipramine, desipramine, trimipramine
Noninterfering: amitriptyline, clobazam, levomepromazine, norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, carbamazepine, citalopram, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine
Interfering: diazepam

KEY WORDS
plasma; clomipramine is IS

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 8

Internal standard: minaprine (5.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: desipramine, imipramine
Also analyzed: diltiazem, nortriptyline, amitriptyline, haloperidol, propafenone, amiodarone, verapamil

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 27
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, doxepin, desipramine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE
Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 3 μL 20 ng/mL clobazam + 1 mL saturated sodium borate (adjusted to pH 11 with 6 M NaOH) + 5 mL n-hexane, mix 2 min, centrifuge at 3000 g for 10 min. Remove organic phase and evaporate to dryness under a stream of helium at 30°. Reconstitute in 20 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long Pelliguard LC-8 40 μm (Supelco)
Column: 150 × 4.6 C8 5 μm (Supelco)

Mobile phase: MeCN:buffer 50:50 (Buffer was 1.2 mL butylamine in 1 L 10 mM NaH_2PO_4, pH adjusted to 3 with phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.085
Internal standard: clobazam (k' 1.344)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: desipramine, nortriptyline, imipramine, amitriptyline
Simultaneous: nitrazepam, lorazepam, clonazepam, triazolam, flunitrazepam, alprazolam, diazepam, haloperidol, maprotiline

KEY WORDS
serum

REFERENCE
Segatti,M.P.; Nisi,G.; Grossi,F.; Mangiarotti,M.; Lucarelli,C. Rapid and simple high-performance liquid chromatographic determination of tricyclic antidepressants for routine and emergency serum analysis, *J.Chromatogr.*, **1991**, *536*, 319–325.

SAMPLE
Matrix: blood
Sample preparation: For each 1 mL plasma or serum add 10 µL 14 µg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 20 × 4.6 10 µm Hypersil MOS C8; B 20 × 4.6 5 µm Hypersil CPS CN + 250 × 4.6 5 µm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K_2HPO_4 adjusted to pH 6.8 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 8.54
Internal standard: trimipramine (6.5)
Limit of detection: 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, desipramine, fluvoxamine, imipramine, maprotiline, nortriptyline
Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol
Interfering: amitriptyline, doxepin

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL protriptyline in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 12.6
Internal standard: protriptyline (6.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)

Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 28.5
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES
Extracted: chlorpromazine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, imipramine, pipamperone, risperidone, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 500 ng/mL imipramine in EtOH + 500 μL 500 mM NaOH, mix, add to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 5 mL n-hexane:isoamyl alcohol 98:2. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Partisphere silica (Whatman)
Mobile phase: Hexane:EtOH:dichloromethane:diethylamine 77:18:5:0.003
Flow rate: 1.3
Detector: UV 214

CHROMATOGRAM
Retention time: 10
Internal standard: imipramine (3)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, desipramine, doxepin, fluoxetine

KEY WORDS
plasma; SPE; normal phase

REFERENCE
Altieri,I.; Pichini,S.; Pacifici,R.; Zuccaro,P. Improved clean-up procedure for the high-performance liquid chromatographic assay of clomipramine and its demethylated metabolite in human plasma, *J.Chromatogr.B*, **1995**, *669*, 416–417.

SAMPLE
Matrix: blood
Sample preparation: Stabilize plasma with 2 mg/mL sodium borohydride to prevent conversion of lofepramine to desipramine. 2 mL Plasma + 200 μL 1 M pH 11 sodium car-

bonate + 5 mL hexane:isoamyl alcohol 99:1, shake for 5 min, centrifuge at 3000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 100 μL mobile phase, inject a 70 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long 5 μm Supelcosil LC-PCN
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:buffer 120:35:100 (Buffer was 2.07 g/L $NaH_2PO_4.H_2O$ in water.)
Flow rate: 2.5
Injection volume: 70
Detector: UV 254

CHROMATOGRAM

Retention time: 3.46
Internal standard: clomipramine

OTHER SUBSTANCES

Extracted: lofepramine, desipramine
Simultaneous: nortriptyline, zuclopenthixol, imipramine, perphenazine, flupentixol, amitriptyline, haloperidol

KEY WORDS

plasma; clomipramine is IS

REFERENCE

Elm,T.; Hansen,E.L. Simultaneous determination of lofepramine and desipramine by a high-performance liquid chromatographic method used for therapeutic drug monitoring, *J.Chromatogr.B*, **1995**, *665*, 355–361.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 252

CHROMATOGRAM

Retention time: 12.00
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 25 μL MeOH, vortex for 30 s, add 100 μL 5 M NaOH, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb MV octadecyl

Mobile phase: MeCN:10 mM triethylamine 60:40, pH adjusted to 3.0 with 85% phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 260

CHROMATOGRAM
Retention time: 8.3
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: desipramine, imipramine

KEY WORDS
mouse; serum; clomipramine is IS

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Dewey,M.J. Rapid microsample analysis of imipramine and desipramine by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1995**, *668*, 338–342.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 μL buffer + 50 μL 8 μg/mL IS in MeOH + 5 mL heptane:ethyl acetate 80:20, mix mechanically for 10 min, centrifuge at 3500 g for 10 min. Remove the organic layer and add it to 1 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3500 g for 10 min. Remove the aqueous layer and add it to 400 μL buffer and 5 mL heptane:ethyl acetate 80:20, extract, centrifuge at 3500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 150 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by adjusting pH of 8% sodium bicarbonate solution to 10.5 with 100 mM NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: MeOH:water 70:30 containing 7 mM butylamine
Flow rate: 1.2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: metabolite I of trimipramine (14)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: chlorpromazine, flunitrazepam, levomepromazine, lorazepam, nitrazepam, oxazepam

KEY WORDS
serum

REFERENCE
Coudore,F.; Hourcade,F.; Molinier-Manoukian,C.; Eschalier,A.; Lavarenne,J. Application of HPLC with silica-phase and reversed-phase eluents for the determination of clomipramine and demethylated and 8-hydroxylated metabolites, *J.Anal.Toxicol.*, **1996**, *20*, 101–105.

SAMPLE
Matrix: blood, erythrocytes
Sample preparation: Evaporate 100 μL 10 μg/mL levallorphan in MeOH into a tube under a stream of nitrogen at 30°, add 1 mL plasma or erythrocytes, add 5 mL hexane:diethyl ether 80:20, shake mechanically for 10 min, centrifuge briefly at 4° at 3000 g, repeat

extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm cyanopropylsilane (Societe SGE)
Mobile phase: MeCN:MeOH:10 mM pH 7.0 potassium phosphate 60:15:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 25
Detector: UV 220

CHROMATOGRAM
Retention time: 7.3
Internal standard: levallorphan (6.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, amitriptyline, carbamazepine, clonazepam, diazepam, digoxin, fluvoxamine, haloperidol, imipramine, oxazepam, phenytoin, prazepam, salicylic acid, theophylline, thioproperazine, thioridazine, triazolam, valproic acid

KEY WORDS
plasma

REFERENCE
Marescaux,P.; Belan,E.; Houdret,N.; Goudemand,M.; Lhermitte,M. Simultaneous determination of clomipramine and its demethylated metabolite in plasma and erythrocytes by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2171–2177.

SAMPLE
Matrix: blood, tissue
Sample preparation: 100 μL Serum or 200 μL brain homogenate + 100 μL 5.0 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 5 min. Evaporate organic layer under a gentle stream of nitrogen at 20°. Reconstitute residue with 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Microsorb MV C18 (Rainin, Woburn, USA)
Mobile phase: MeCN:water 55:45 containing 10 mM triethylamine, pH adjusted to 4.8 with 85% phosphoric acid
Flow rate: 1.0
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Internal standard: clomipramine

KEY WORDS
serum; brain; mouse; clomipramine is IS

REFERENCE
Holladay,J.W.; Dewey,M.J.; Yoo,S.D. Pharmacokinetics and antidepressant activity of fluoxetine in transgenic mice with elevated serum α-1-acid glycoprotein levels, *Drug Metab.Dispos.*, **1998**, *26*, 20–24.

SAMPLE
Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 32.1
Internal standard: cianopramine (8.93)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, tissue

Sample preparation: Whole blood. 1 mL Whole blood + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, agitate gently for 30 min, centrifuge at 3500 rpm for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min, inject a 30 μL aliquot of the aqueous layer. Liver. Homogenize 10 g freshly minced liver with 10 mL water, adjust pH to 10 with 1 M NaOH, add 10 mg subtilisin (Sigma), heat at 55° for 1 h, adjust pH to 7.0 ± 0.5 with dilute mineral acid. 500 μL Homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, agitate gently, centrifuge for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate, centrifuge, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeCN:buffer 40:60 (Buffer was 100 mM potassium phosphate containing 2.5% diethylamine, pH 8.0.)
Flow rate: 2
Injection volume: 30
Detector: UV 220, UV 254

CHROMATOGRAM

Retention time: 32
Internal standard: cianopramine (8.9 ?)
Limit of detection: <200 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Cordner,S.M.; Drummer,O.H. Postmortem clomipramine: therapeutic or toxic concentrations?, *J.Forensic Sci.*, **1994**, *39*, 486–493.

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 20.6 (A), 40.2 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μL Urine + 470 μL 3% L-(+)-ascorbic acid in 200 mM KH_2PO_4 + 30 μL β-glucuronidase/arylsulfatase (Boehringer Mannheim), vortex for 2 s, heat at 37° for 16 h to deconjugate, add 50 μL 2 M NaOH. 1 mL Plasma, urine, or deconjugated urine + 1 mL 600 mM pH 11.3 potassium carbonate + 100 μL 5 (plasma) or 20 (urine) μM desipramine in EtOH + 5 mL heptane:MTBE:n-butanol 47.5:47.5:5, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze at -50°. Remove the organic layer and add it to 1 mL 20 mM HCl, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze. Discard the organic layer and add 500 μL 600 mM pH 11.3 potassium carbonate to the thawed aqueous layer. Add 3 mL heptane:MTBE:n-butanol 47.5:47.5:5 to the aqueous layer, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeOH, vortex for 5 s, centrifuge at 1400 g for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4 7 μm 120 Å Nucleosil

Column: 250 × 4 5 μm 100 Å Nucleosil RP-phenyl

Mobile phase: MeCN:buffer 30:70 (Buffer was 14.05 g sodium perchlorate + 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 25.33

Internal standard: desipramine (14.19)

Limit of detection: 10 nM (urine), 5 nM (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Nielsen,K.K.; Brosen,K. High-performance liquid chromatography of clomipramine and metabolites in human plasma and urine, *Ther.Drug Monit.*, **1993**, *15*, 122–128.

SAMPLE

Matrix: blood, urine

Sample preparation: 100 μL Serum or urine + 25 μL MeOH + 100 μL 5 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 5000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 10 × 4.6 5 μm Microsorb MV C18

Mobile phase: MeCN:10 mM triethylamine in water 60:40, pH adjusted to 3.0 with 85% phosphoric acid

Flow rate: 1

Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: desipramine, imipramine

KEY WORDS
mouse; serum; clomipramine is IS

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Baumann,H.; Dewey,M.J. Altered disposition and antidepressant activity of imipramine in transgenic mice with elevated α-1-acid glycoprotein, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 918–922.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.442

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract

with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, dothiepin, doxepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluororacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 8.3

OTHER SUBSTANCES
Simultaneous: cimetidine, halofantrine, haloperidol, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6

Injection volume: 20
Detector: UV 265

OTHER SUBSTANCES

Also analyzed: amitriptyline, chlorpromazine, promazine, promethazine, thymol

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, prom-

azine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 17.90 (A), 8.53 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin,

trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

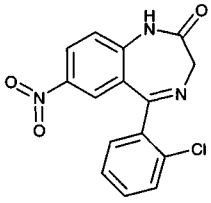
CHROMATOGRAM
Retention time: k' 3.99

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Clonazepam



Molecular formula: $C_{15}H_{10}ClN_3O_3$
Molecular weight: 315.72
CAS Registry No.: 1622-61-3
Merck Index: 2449

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.3 (A), 18.2 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 μL 512 nM IS in MeOH:water 5:95, add to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH. Elute with 200 μL and 100 μL MeOH, evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 4 μm Novapak C18
Mobile phase: MeCN:MeOH:10 mM pH 3.7 K_2HPO_4 30:2:10
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 7.4
Internal standard: flunitrazepam (9.8)
Limit of detection: 5 nM

OTHER SUBSTANCES
Extracted: alprazolam, nitrazepam
Simultaneous: amobarbital, carbamazepine, citalopram, clobazam, clozapine, diazepam, doxepin, ethosuximide, norclobazam, oxazepam, oxcarbamazepine, pentobarbital, phenobarbital, phenytoin, primidone, valproic acid, zopiclone
Interfering: medazepam, midazolam, nordiazepam, temazepam

KEY WORDS
SPE; plasma; serum

REFERENCE
Åkerman,K.K.; Jolkkonen,J.; Parviainen,M.; Penttilä,I. Analysis of low-dose benzodiazepines by HPLC with automated solid-phase extraction, *Clin.Chem.*, **1996**, *42*, 1412–1416.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 17.417

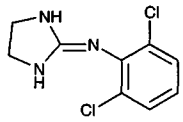
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clonidine



Molecular formula: $C_9H_9Cl_2N_3$
Molecular weight: 230.10
CAS Registry No.: 4205-90-7, 4205-91-8 (HCl)
Merck Index: 2450
Lednicer No.: 1 241, 4 5, 38, 88

SAMPLE

Matrix: blood
Sample preparation: 50 µL Serum + 50 µL 10 mM HCl + 50 µL 3 M NaOH + 1.5 mL n-butyl chloride:isopropanol 95:5, vortex 60 s, centrifuge at 2500 rpm (20 cm) for 2 min. Freeze in dry ice/isopropanol for 20 s and remove organic phase. Add 100 µL 50 mM phosphoric acid to organic phase, vortex 60 s, centrifuge at 2500 rpm (20 cm) for 2 min, remove organic layer, inject 25 µL aliquots of aqueous layer.

HPLC VARIABLES

Guard column: 20 × 4.6 5 µm Supelcosil LC-PCN
Column: 150 × 4.6 5 µm Supelcosil LC-PCN
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KOH/H_3PO_4, pH 3.0.)
Column temperature: 35
Flow rate: 2
Injection volume: 25
Detector: UV 214

CHROMATOGRAM

Retention time: 4.4
Internal standard: clonidine

OTHER SUBSTANCES

Simultaneous: tolazoline
Noninterfering: amikacin, ampicillin, dopamine, furosemide, gentamicin, heparin, morphine, pancuronium

KEY WORDS

serum; clonidine is IS

REFERENCE

Todesco,L.M.; Thoma,J.J.; Barth,R.D.; Myers,N.J.; White,R.; Ward,R.M. Quantitative determination of tolazoline in human serum by high performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 78–84.

SAMPLE

Matrix: blood
Sample preparation: Add 125 mg NaF to each 1 mL of blood collected. 500 µL Plasma + 500 µL water + 3 mL dichloromethane, shake gently for 10 min, centrifuge at 2100 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 µL 1 mM HCl, vortex for 4 min, sonicate for 45 s, add 2 mL diethyl ether, vortex for 2 min, centrifuge at 2100 g for 5 min, discard the ether phase, apply a vacuum to the aqueous phase for 10 s, inject a 35-100 µL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 15 × 4.6 37 µm Corasil C18
Column: 150 × 4.6 5 µm Spherisorb ODS-1

Mobile phase: MeCN:MeOH:7 mM pH 4.0 potassium phosphate buffer 30:15:55
Flow rate: 1.2
Injection volume: 35-100
Detector: UV 214

CHROMATOGRAM
Retention time: 12
Internal standard: clonidine

OTHER SUBSTANCES
Extracted: pentobarbital, pilocarpine

KEY WORDS
plasma; human; dog; clonidine is IS

REFERENCE
Weaver,M.L.; Tanzer,J.M.; Kramer,P.A. High-performance liquid chromatographic determination of pilocarpine in plasma, *J.Chromatogr.*, **1992**, *581*, 293–296.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 3.96
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam;

amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 6.128

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Grind 2 tablets, sonicate in 15 mL water for 15 min, shake vigorously for 30 min, add 25 mL MeOH, shake 1 h, dilute to 50 mL with MeOH, mix, centrifuge, filter (1 μm or smaller), inject 25 μL aliquot

HPLC VARIABLES

Column: 250 × 4.6 trimethylsilyl chloride bonded to 5-6 μm spherical silica

Mobile phase: MeOH:buffer 65:35 (Buffer was 2.2 mM KH_2PO_4 + 16 mM Na_2HPO_4, pH 7.9.)

Flow rate: 1

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: chlorthalidone

KEY WORDS

tablets

REFERENCE

Walters,S.M.; Stonys,D.B. Determination of chlorthalidone and clonidine hydrochloride in tablets by HPLC, *J.Chromatogr.Sci.*, **1983**, *21*, 43–45.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, shake with 25 mL water or 80:20 MeOH:water for 30 min, filter. Dilute eye drops 100 times with MeOH:water 80:20

HPLC VARIABLES

Column: 125 × 4.6 Nucleosil 5 C18

Mobile phase: MeOH:water:triethylamine 80:20:0.005

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 3

KEY WORDS

tablets; eye drops

REFERENCE

Wilczynska-Wojtulewicz,I.; Sadlej-Sosnowska,N. Determination of clonidine hydrochloride in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *367*, 434–437.

SAMPLE

Matrix: formulations

Sample preparation: Dilute a 500 μL aliquot of syrup with water to give a clonidine concentration of 5 μg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax TMS trimethylsilyl
Mobile phase: MeOH:buffer 65:35 (Buffer was 2.2 mM KH_2PO_4 and 16 mM Na_2HPO_4, pH 7.9.)
Flow rate: 1
Injection volume: 15
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5
Internal standard: guanabenz acetate (7)

KEY WORDS

syrup; stability-indicating

REFERENCE

Levinson,M.L.; Johnson,C.E. Stability of an extemporaneously compounded clonidine hydrochloride oral liquid, *Am.J.Hosp.Pharm.*, **1992**, *49*, 122–125.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine,

methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 1

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, chlorpheniramine, diphenhydramine, promethazine, pyrilamine, triprolidine

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REFERENCE

Supelco Catalog, **1994**, 768.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 μm silica (Phenomenex)
Mobile phase: MeCN:6.25 mM pH 3.0 phosphate buffer 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.61

OTHER SUBSTANCES
Also analyzed: atenolol, diltiazem, metoprolol, nifedipine, prazosin, propranolol, verapamil

REFERENCE
Simmons,B.R.; Stewart,J.T. HPLC separation of selected cardiovascular agents on underivatized silica using an aqueous organic mobile phase, *J.Liq.Chromatogr.*, **1994**, *17*, 2675–2690.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.57

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.97 (A), 4.25 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.14

OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

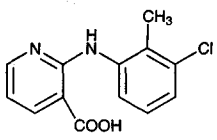
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Clonixin



Molecular formula: $C_{13}H_{11}ClN_2O_2$
Molecular weight: 262.70
CAS Registry No.: 17737-65-4, 55837-30-4 (lysine salt)
Merck Index: 2453
Lednicer No.: 2 281

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

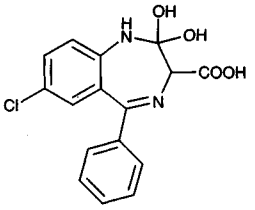
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, pro-

gesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Clorazepate



Molecular formula: $C_{16}H_{13}ClN_2O_4$
Molecular weight: 332.75
CAS Registry No.: 20432-69-3, 57109-90-7 (dipotassium salt), 5991-71-9 (monopotassium slat)
Merck Index: 2465

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL buffer, vortex, add 10 mL 60 ng/mL diazepam in hexane:ethyl acetate 70:30, shake for 10 min, centrifuge at 320 g for 5 min. Remove 8 mL of the organic phase and evaporate it to dryness at 38° under vacuum, dissolve the residue in 500 μL ethyl acetate, vortex for 20 s, evaporate to dryness, reconstitute in 100 μL mobile phase, inject a 50 μL aliquot. (Buffer was 2 M glycine adjusted to pH 9.0 with 1 m NaOH.)

HPLC VARIABLES

Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 45:55 (Buffer was 800 mL 62.5 mM sodium acetate adjusted to pH 5.0 with 1 M NaOH and made up to 1 L.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 2.1
Internal standard: diazepam (9.2)
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: flurazepam, pentobarbital, cimetidine, oxazepam, nordiazepam, prazepam

KEY WORDS

plasma; human; dog

REFERENCE

Colin,P.; Sirois,G.; Lelorier,J. High-performance liquid chromatography determination of dipotassium clorazepate and its major metabolite nordiazepam in plasma, *J.Chromatogr.*, **1983**, *273*, 367–377.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 2 mL water + 50 μL 3.2 μg/mL estazolam in MeOH + 2 mL 100 mM NaOH, mix gently, add 8 mL diethyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 Shim-pack FLC-C8 (Shimadzu)
Mobile phase: MeOH:buffer 53:47 (Buffer was 5 mM Na_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Flow rate: 0.6
Injection volume: 50

Detector: UV 254

CHROMATOGRAM
Retention time: 5.3
Internal standard: estazolam (4)

OTHER SUBSTANCES
Extracted: diazepam, nordiazepam, triazolam
Simultaneous: sulpride, bromazepam, nitrazepam, flunitrazepam
Noninterfering: haloperidol, trihexyphenidyl
Interfering: temazepam, oxazepam

KEY WORDS
serum

REFERENCE
Tada,K.; Moroji,T.; Sekiguchi,R.; Motomura,H.; Noguchi,T. Liquid-chromatographic assay of diazepam and its major metabolites in serum, and application to pharmacokinetic study of high doses of diazepam in schizophrenics, *Clin.Chem.*, **1985**, *31*, 1712–1715.

SAMPLE
Matrix: blood
Sample preparation: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES
Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm
Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 230

CHROMATOGRAM
Retention time: 24

OTHER SUBSTANCES
Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clotiazepam, desmethylclobazam, diazepam, estazolam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam
Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid
Interfering: desmethyldiazepam

KEY WORDS
plasma; column-switching

REFERENCE
Lacroix,C.; Wojciechowski,F.; Danger,P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 229

CHROMATOGRAM

Retention time: 5.19

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine;

ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 227.5

CHROMATOGRAM

Retention time: 18.44

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Capsules. Dissolve contents of 4 capsules in 200 mL 10 mM NaOH with sonication for 5 min, make up to 250 mL with 10 mM NaOH, filter (0.4-0.5 μm), if necessary dilute with 10 mM NaOH to obtain a concentration of about 50 μg/mL clorazepate. Dilute 5 mL of this solution + 2 mL 300 μg/mL dimethylaniline hydrochloride in 10 mM NaOH to 10 mL with 10 mM NaOH, inject a 20 μL aliquot. Tablets. Homogenize tablets in 200 mL 10 mM NaOH at 6000 rpm for 3 min, filter (0.4-0.5 μm), if necessary dilute with 10 mM NaOH to give a concentration of 50-60 μg/mL. Dilute 5 mL of this solution + 2 mL 300 μg/mL dimethylaniline hydrochloride in 10 mM NaOH to 10 mL with 10 mM NaOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak C18

Mobile phase: MeCN: 5 mM tetra-n-butylammonium hydroxide, pH adjusted to 7.5 with phosphoric acid 30:70
Flow rate: 1.8
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6
Internal standard: 2,6-dimethylaniline hydrochloride (10)

OTHER SUBSTANCES
Simultaneous: nordiazepam, degradation products

KEY WORDS
capsules; tablets; stability-indicating

REFERENCE
Elrod,L.,Jr.; Shada,D.M.; Taylor,V.E. High-performance liquid chromatographic analysis of clorazepate dipotassium and monopotassium in solid dosage forms, *J.Pharm.Sci.*, **1981**, *70*, 793–795.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine,

methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova pak C18
Mobile phase: MeCN:water 57:43
Column temperature: 44
Flow rate: 1.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10.7

OTHER SUBSTANCES

Simultaneous: bromazepam, chlordiazepoxide, clobazam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

REFERENCE

Guillaume,Y.; Guinchard,C. Marked difference between acetonitrile/water and methanol/water mobile phase systems on the thermodynamic behavior of benzodiazepines in reversed phase liquid chromatography, *Chromatographia*, **1995**, *41*, 84–87.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova pack C18
Mobile phase: MeOH:water 52:48
Column temperature: 48

Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 12.5

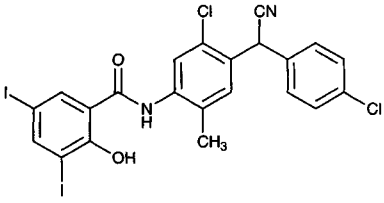
OTHER SUBSTANCES

Simultaneous: bromazepam, chlordiazepoxide, clobazam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

REFERENCE

Guillaume,Y.; Guinchard,C. Thermodynamic behavior of mixed benzodiazepines by a new liquid chromatographic method, *Chromatographia*, **1995**, *40*, 193–196.

Closantel



Molecular formula: $C_{22}H_{14}Cl_2I_2N_2O_2$
Molecular weight: 663.08
CAS Registry No.: 57808-65-8
Merck Index: 2473

SAMPLE

Matrix: blood, tissue

Sample preparation: Condition a 250mg Bond Elut C18 (18% carbon loaded) SPE cartridge with 5 mL MeCN and 5 mL MeCN:water 10:90 containing 0.1% diethylamine. Mix 3 mL plasma or 3 g ground tissue with 3 mL 0.05% acetic acid in water saturated with NaCl and 10 mL MeCN using a high-speed blender for 3 min, centrifuge at 2400 g for 5 min. Dilute supernatant with 25 mL MeCN, centrifuge, concentrate to 2-3 mL at 50° under vacuum. Add to the SPE cartridge, elute with 5 mL MeCN, concentrate the eluate to 300 µL, inject a 30 µL aliquot.

HPLC VARIABLES

Column: 125 × 4 5µm LiChrospher 100 RP18 ODS1
Mobile phase: MeCN:water 85:15, adjusted to pH 2.5 with phosphoric acid
Flow rate: 1
Injection volume: 30
Detector: UV 254; F ex 335 em 510

CHROMATOGRAM

Retention time: 7.7
Limit of detection: 200-500 µg/kg (UV); 10-50 µg for F

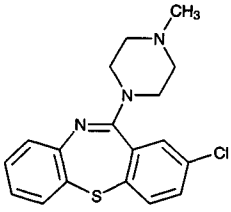
KEY WORDS

fat; kidney; liver; muscle; SPE

REFERENCE

Stoev,G. Determination of closantel residues in plasma and tissues by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1998**, *710*, 234–238.

Clothiapine



Molecular formula: $C_{18}H_{18}ClN_3S$
Molecular weight: 343.88
CAS Registry No.: 2058-52-8
Merck Index: 2476

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL MeOH + 1 M NaOH, extract with 6 mL hexane:isopropanol 99:1 by rotary mixing for 10 min, centrifuge at 2000 g for 5 min. Add 300 μL 200 mM HCl to the organic layer, mix, centrifuge at 2000 g for 5 min, discard the upper organic layer, inject a 120 μL aliquot of the acidic aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 Spherisorb C8
Mobile phase: MeCN:triethylamine:Pic B5:60 mM pH 6.4 phosphate buffer 48:0.05:0.15:52, adjusted to pH 6.4 with orthophosphoric acid
Flow rate: 1.6
Injection volume: 120
Detector: UV 214

CHROMATOGRAM

Retention time: 9.6
Internal standard: clothiapine

OTHER SUBSTANCES

Extracted: buprenorphine
Simultaneous: amitriptyline, amoxapine, carpipramine, clomipramine, demexiptilline, desipramine, diazepam, dosulepin, doxepin, imipramine, maprotiline, medifoxamine, metapramine, methadone, mianserine, naloxone, normaprotiline, nortriptyline, opipramol, oxaflozane, paroxetine, quinupramine, tianeptine, trazodone, viloxazine
Noninterfering: codeine, pholcodeine, codethyline, morphine
Interfering: clomipramine

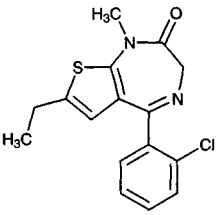
KEY WORDS

plasma; clothiapine is IS

REFERENCE

Lagrange,F.; Pehourcq,F.; Baumvieille,M.; Begaud,B. Determination of buprenorphine in plasma by liquid chromatography: application to heroin-dependent subjects, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1295–1300.

Clotiazepam



Molecular formula: $C_{16}H_{15}ClN_2OS$
Molecular weight: 318.83
CAS Registry No.: 33671-46-4
Merck Index: 2477

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM

Retention time: 21.652

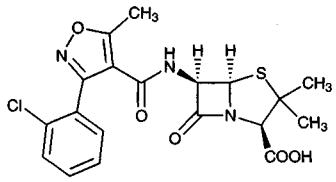
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cloxacillin



Molecular formula: $C_{19}H_{18}ClN_3O_5S$
Molecular weight: 435.89
CAS Registry No.: 61-72-3, 23736-58-5 (benzathine), 7081-44-9 (sodium salt, monohydrate), 642-78-4 (sodium salt)
Merck Index: 2480
Lednicer No.: 1 413

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 28:72 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Retention time: 6.2
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood
Sample preparation: 400 μL Serum + 400 μL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 μL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: dicloxacillin, methicillin, nafcillin, oxacillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefamandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS
serum

REFERENCE
Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 15 μL 500 μg/mL nafcillin + 50 μL 1 M sulfuric acid, mix for 30 s, add 2 mL dichloromethane, mix for 2 min, centrifuge at 1130 g for 2 min. Remove 1.8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 20:80
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Internal standard: nafcillin (4)
Limit of quantitation: 10 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Jamaluddin,A.B.M.; Sarwar,G.; Rahim,M.A.; Rahman,M.K. Assay for cloxacillin in human serum utilising high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *490*, 243–246.

SAMPLE
Matrix: blood
Sample preparation: Condition a 55 × 5 100-200 mesh AG 50W-X8 (H^+) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 μL Serum + 600 μL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 μL effluent, collect the rest of the effluent. Remove a 450 μL aliquot and add it to 50 μL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 μL aliquot and add it to 15 μL 200 mM pH 6.0 phosphate buffer, add 35 μL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 μL 1 M HCl, inject a 5-10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM pH 3.0 phosphate buffer 55:45
Flow rate: 1
Injection volume: 5-10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 7
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: dicloxacillin

KEY WORDS
derivatization; serum; SPE

REFERENCE
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

SAMPLE
Matrix: blood, milk
Sample preparation: Milk. Adjust 5 mL milk containing 250 ng IS to pH 6.3 with 100 mM HCl, deproteinize with 10 mL MeCN. Centrifuge at 1932 g for 20 min and extract the aqueous phase with two 5 mL portions of chloroform for 20 min (Caution! Chloroform is a carcinogen!). Centrifuge at 1932 g for 20 min. Evaporate the organic phase to dryness, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot. Serum. Adjust 2.5 mL serum containing 250 ng IS to pH 6.3 with 100 mM HCl, deproteinize with 10 mL MeCN. Centrifuge at 1932 g for 20 min and extract the aqueous phase with two 5 mL portions of dichloromethane for 20 min. Centrifuge at 1932 g for 20 min. Evaporate the organic phase to dryness, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot

HPLC VARIABLES
Column: 15 × 3.9 4 μm Nova Pack C18
Mobile phase: MeCN:20 mM KH_2PO_4 21:79, pH 5
Flow rate: 1.2
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Retention time: 5.6
Internal standard: oxacillin (3.8)
Limit of quantitation: 10 ng/mL (milk), 50 ng/mL (blood)

KEY WORDS
serum; cow

REFERENCE
Pérez,B.; Prats,C.; Castells,E.; Arboix,M. Determination of cloxacillin in milk and blood of dairy cows by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *698*, 155–160.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma, serum. 100 μL Plasma or serum + oxacillin + 100 μL 500 mM pH 2.2 citric acid buffer + 20 μL 500 mM HCl + 2.5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in mobile phase, inject an aliquot. Urine. Dilute urine with water, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 ODS pellicular
Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:20 mM pH 6.6 sodium acetate 34:100
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 9
Internal standard: oxacillin (7)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dicloxacillin, flucloxacillin

KEY WORDS
plasma; serum; pharmacokinetics

REFERENCE
Thijssen,H.H.W. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 339–345.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 50 μL 240 μg/mL nafcillin + 50 μL 1 M sulfuric acid, mix for 5 s, add 2 mL dichloromethane, shake for 2 min, centrifuge at 2000 rpm for 2 min. Remove the organic layer and add it to 1 mL 40 mM pH 6.8 NaH_2PO_4, shake for 2 min, centrifuge at 2000 rpm for 2 min. Place the aqueous layer in a separate tube, agitate to remove traces of dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 100 × 4.6 10 μm MPLC Concept RP-8 (Brownlee)
Mobile phase: MeCN:40 mM NaH_2PO_4 6.2:20, pH 4.5
Flow rate: 1.6
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 5.10
Internal standard: nafcillin (6.60)
Limit of detection: 50 ng/mL

KEY WORDS
serum

REFERENCE
Teare,F.W.; Kwan,R.H.; Spino,M.; MacLeod,S.M. High-pressure liquid chromatographic assay of cloxacillin in serum and urine, *J.Pharm.Sci.*, **1982**, *71*, 938–941.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.702

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk, formulations
Sample preparation: Weigh out bulk drug, capsule contents, injections, or syrup containing 50 mg cloxacillin, add 500 μL 30 mg/mL dimethyl phthalate in MeCN:water 50:50, make up to 50 mL with water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:4% acetic acid 60:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5
Internal standard: dimethyl phthalate (3.7)

KEY WORDS

injections; syrup; capsules

REFERENCE

Hsu,M.-C.; Cheng,M.-C. High-performance liquid chromatographic method for the determination of cloxacillin in commercial preparations and for stability studies, *J.Chromatogr.*, **1991**, *549*, 410–415.

SAMPLE

Matrix: formulations

Sample preparation: Capsules. Dissolve the powder from five capsules and the capsule shells in water, dilute to 1 L with water, filter an aliquot of the solution (0.2 μm), dilute 10-fold with water, inject an aliquot. Syrup. Reconstitute the syrup powder, dilute 200-fold with water, filter, inject an aliquot. Neonatal suspension. Reconstitute the powder, dilute 800-fold with water, filter, inject an aliquot.

HPLC VARIABLES

Column: 100 × 2.0 3 μm Hypersil ODS

Mobile phase: MeCN:20 mM phosphate buffer 15:85 containing 100 mM sodium dodecyl sulfate, adjusted to pH 2.0 with orthophosphoric acid

Flow rate: 0.4

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: degradation products, ampicillin

KEY WORDS

capsules; suspensions; syrup

REFERENCE

Shakoor,O.; Taylor,R.B. Analysis of ampicillin, cloxacillin and their related substances in capsules, syrups and suspensions by high-performance liquid chromatography, *Analyst*, **1996**, *121*, 1473–1477.

SAMPLE

Matrix: formulations

Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 70 mm long Co:Pell ODS

Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)

Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 14.4

Limit of detection: 1890 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin, ampicillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V

KEY WORDS

tablets; capsules; oral suspensions; injections

REFERENCE

Briguglio,G.T.; Lau-Cam,C.A. Separation and identification of nine penicillins by reverse phase liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 228–231.

SAMPLE

Matrix: milk

Sample preparation: Condition a 3 mL 500 mg Baker-10 C18 SPE cartridge (J.T. Baker) with 3 mL MeOH and 3 mL distilled water. Add 20 mL MeCN to 10 mL milk, vortex for 1 min, centrifuge at 1500 g for 10 min, concentrate the supernatant to 2-3 mL on a rotary evaporator at 40°, add to the SPE cartridge, dry the cartridge under reduced pressure for 3 min, elute with 1 mL MeOH, filter (0.45 μm) the eluate, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Kaseisorb LC ODS-300-5 (Tokyo Kasei)

Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 buffer 20:10:80 containing 5 mM sodium 1-decanesulfonate, adjusted to pH 3.5 with concentrated phosphoric acid

Column temperature: 40

Flow rate: 1

Injection volume: 10

Detector: UV 210

CHROMATOGRAM

Retention time: 16.5

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: ampicillin, dicloxacillin, nafcillin, penicillin G

KEY WORDS

SPE

REFERENCE

Takeba,K.; Fujinuma,K.; Miyazaki,T.; Nakazawa,H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 205–211.

SAMPLE

Matrix: milk

Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 10 mL MeOH, 10 mL water, 5 mL 2% NaCl, and 5 mL 100 mM pH 8 phosphate extraction buffer. Add 30 mL 100 mM pH 8 phosphate extraction buffer to 5 mL milk, add 1.65 mL 1 M sulfuric acid to reach pH 4.0-4.5, vortex for 30 s, centrifuge at 2400 g for 10 min, add 600 μL 5 M NaOH to the supernatant to reach pH 8, vortex, centrifuge at 2400 g for 5 min. Add the supernatant to a reservoir attached to the SPE cartridge, pull through the SPE cartridge at 3 mL/min, remove the reservoir and elute with 1 mL MeCN:water 40:60. Add 500 μL derivatizing reagent to the eluate, vortex, heat at 65° for 10 min, cool to room temperature (protect from light), inject a 100 μL aliquot of the derivatized sample. (Prepare the 100 mM pH 8 phosphate extraction buffer as follows. Dissolve 15.6 g K_2HPO_4 dihydrate in 800 mL water, adjust pH to 8 with 10 M NaOH, make up to 1 L. Prepare the derivatizing reagent as follows. Weigh out 13.78 g 1,2,4-triazole, add 60 mL water, stir, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, dilute to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)

Mobile phase: MeCN:MeOH:buffer 37:5:58 (Prepare the 100 mM pH 6.5 phosphate buffer containing 15 mM thiosulfate and 30 mM tetrabutylammonium hydrogen sulfate as follows. Weigh 4.969 g anhydrous NaH_2PO_4, 10.139 g Na_2HPO_4 dihydrate, 3.894 g sodium thiosulfate pentahydrate, and 10.186 g tetrabutylammonium hydrogen sulfate, dissolve in 800 mL water, adjust pH to 6.5 with 5 M NaOH, dilute to 1 L with water, mix thoroughly, filter under vacuum (0.45 μm).)

Flow rate: 1

Injection volume: 100
Detector: UV 340

CHROMATOGRAM
Retention time: 12
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: oxacillin, dicloxacillin

KEY WORDS
derivatization; SPE

REFERENCE
Verdon,E.; Couedor,P. Determination of isoxazolylpenicillins residues in milk by ion-pair reversed-phase high-performance liquid chromatography after precolumn derivatization, *J.Chromatogr.B*, **1998**, *705*, 71–78.

SAMPLE
Matrix: milk
Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 µL aliquot. (Prepare IS solution by dissolving 10 µL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 µm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 8.18
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Simultaneous: penicillin G, methicillin, oxacillin, dicloxacillin, penicillin V
Interfering: nafcillin, phenethicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 500 μL MeCN:MeOH:water 40:20:40, vortex for 10-15 s, filter (Centricon-10, molecular mass cut-off filter 10000 daltons) with centrifuging at 2677 g for 30 min, inject a 10-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 phenyl microbore (UV detection) or 220 × 4.6 5 μm Spheri-5 phenyl microbore (MS detection)
Mobile phase: MeCN:85% phosphoric acid:water 27.5:0.1:72.4 containing 1.5 mM octanesulfonate and 2.5 mM dodecanesulfonate (UV) or isopropanol:acetic acid in 200 mM ammonium acetate:water 15:2:83 (MS)
Column temperature: 60
Flow rate: 0.2-0.45 (UV) or 0.8-1.2 (MS)
Injection volume: 10-100
Detector: UV 220 or MS, Finnigan MAT 4800 quadrupole, thermospray, source 320°, vaporizer 120°, pulsed positive ion negative ion

CHROMATOGRAM
Retention time: 12.40 (UV), 8.5 (MS)
Limit of detection: 100 ng/mL (MS), 50 ng/mL (100)

OTHER SUBSTANCES
Also analyzed: ampicillin, amoxicillin

KEY WORDS
ultrafiltrate; LC-MS

REFERENCE
Voyksner,R.D.; Tyczkowska,K.L.; Aronson,A.L. Development of analytical methods for some penicillins in bovine milk by ion-paired chromatography and confirmation by thermospray mass spectrometry, *J.Chromatogr.*, **1991**, *567*, 389–404.

SAMPLE
Matrix: milk
Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES
Column: 100 × 8 Radial-Pak 10 μm μBondapak C18

Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 16
Limit of detection: 0.3 ppm

OTHER SUBSTANCES
Simultaneous: penicillin V, ampicillin, methicillin, penicillin G, oxacillin, nafcillin, dicloxacillin.

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: milk
Sample preparation: 10 mL Milk + 2 mL 200 mM tetraethylammonium chloride, stir, slowly add 38 mL MeCN over 30 s, let stand for 5 min, decant the supernatant through a plug of glass wool. 40 mL Filtrate + 1 mL water, evaporate under reduced pressure to 1-2 mL, make up to 4 mL with water, filter (0.45 μm polyvinylidene difluoride). Inject 2 mL into an LC system (150 × 4.6 5 μm Supelcosil LC-18; MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 40:60 over 27 min, to 0:100 over 1 min; 1 mL/min; UV 210 and 295), collect a 1.5 mL fraction at retention time for cloxacillin (27.5 min), evaporate to 1 mL, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 2 mM phosphoric acid and 8 mM KH_2PO_4.)
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES
Also analyzed: ampicillin, amoxicillin, cephapirin, penicillin G, ceftiofur, penicillin V

KEY WORDS
cow

REFERENCE
Moats,W.A.; Harik-Khan,R. Liquid chromatographic determination of β-lactam antibiotics in milk: A multiresidue approach, *J.AOAC Int.*, **1995**, *78*, 49–54.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a

stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM

Retention time: 45.12

OTHER SUBSTANCES

Extracted: ampicillin, ceftiofur, cephapirin, dicloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS

SPE

REFERENCE

Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE

Matrix: milk

Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM
Retention time: 35.5
Internal standard: penicillin V (28.5)
Limit of detection: 1.4 ng/mL
Limit of quantitation: 1.9 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin, ampicillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS
derivatization; cow; SPE

REFERENCE
Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE
Matrix: milk, tissue
Sample preparation: Milk. Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound in a tube containing 100 μL Na_2HPO_4 (ca. 28.6 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. Tissue. Blend 5 g tissue, 5 mL water, 2 mL 100 mM tetraethylammonium chloride (for liver and kidney 1 mL 200 mM tetraethylammonium chloride and 1 mL 5 mM KH_2PO_4), and 40 mL MeCN at half power for 1 min, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate (20 mL for liver and kidney), add 2 mL buffer, add 5 mL water, add 5 mL t-butanol, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Proceed as above. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (milk) or 40:60 (tissue) (Buffer was 2 mM phosphoric acid containing 8 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

KEY WORDS
muscle; liver; kidney

REFERENCE

Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE

Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: dicloxacillin, methicillin, penicillin G, penicillin V, nafcillin, carbenicillin

REFERENCE

Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE

Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES

Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES
Simultaneous: carbenicillin, cephapirin, dicloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 30:70
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: phenethicillin

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: tissue
Sample preparation: Blend 15 g tissue with 45 mL (60 mL for liver and kidney) water in a 300 or 500 mL blender jar at half power (or less to control foaming) for 2 min. Add a 20 mL aliquot of homogenate to 40 mL MeCN, mix, after 5 min decant supernatant through a plug of glass wool, collect 30 mL. Shake vigorously 30 mL filtrate, 10 mL 200 mM phosphoric acid, and 20 mL dichloromethane, remove organic layer and extract aqueous layer with 10 mL dichloromethane (and 10 mL MeCN for liver and kidneys). Combine dichloromethane layers, add 15 mL MeCN, add 40 mL hexane, wash the mixture twice with 4 mL portions of water, extract the organic layer four times with 1 mL 10 mM pH 7 buffer. Combine extracts and add 0.1-0.2 mL tert-butanol, place in a rotary evaporator without heating at first. When the flask becomes cold warm to 50°, concentrate to less than 1 mL, adjust to a final volume of 1 mL, filter (Gelman Acrodisc LCPVDF), inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: Polymer Labs guard cartridge
Column: 150 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Labs)
Mobile phase: MeCN:buffer 22:78, after run was over flush at 35:65 for 5 min then re-equilibrate with 22:78 for 9 min. (Buffer was 10 mM pH 7 phosphate buffer prepared from 1.36 KH_2PO_4 and 2.84 g Na_2HPO_4 in 3 L water.)
Flow rate: 1
Injection volume: 200

Detector: UV 210

CHROMATOGRAM
Retention time: 9-11
Limit of detection: 10 ng/g

KEY WORDS
cow; pig

REFERENCE
Moats,W.A. High-performance liquid chromatographic determination of penicillin G, penicillin V and cloxacillin in beef and pork tissues, *J.Chromatogr.*, **1992**, *593*, 15–20.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 20 mL 2% NaCl. Shake 10 g tissue and 20 mL MeCN on a mechanical shaker for 30 min, centrifuge, remove the supernatant, repeat the extraction with 20 and 10 mL portions of MeCN. Combine the extracts and add them to 30 mL 4% NaCl, remove the MeCN under reduced pressure at 40°, filter (Whatman GF/C and Gelman 0.45 μm membrane) the remaining aqueous mixture, add the filtrate to the SPE cartridge at <2 mL/min, wash with 15 mL 2% NaCl, elute with 5 mL MeCN. Add 100 μL 20 μg/mL penicillin V in MeCN to the eluate, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 1 mL water, vortex, add 1 mL 2 M pH 9 1,2,4-triazole containing 1 mM mercuric chloride, vortex, heat at 65° for 30 min, cool, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:buffer 22.5:77.5 (Prepare buffer by dissolving 4.96 g Na_2HPO_4, 10.14 g $NaH_2PO_4.2H_2O$, and 3.90 g sodium thiosulfate in 1 L water.)
Flow rate: 1.2
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 23
Internal standard: penicillin V (6.5)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: penicillin G

KEY WORDS
derivatization; cow; sheep; kidney; liver; muscle; SPE

REFERENCE
Gee,H.-E.; Ho,K.-B.; Toothill,J. Liquid chromatographic determination of benzylpenicillin and cloxacillin in animal tissues and its application to a study of the stability at -20°C of spiked and incurred residues of benzylpenicillin in ovine liver, *J.AOAC Int.*, **1996**, *79*, 640–644.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to

stand for 30 min. Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 μL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES

Column: A 4 × 4 5 μm LiChrospher 100 RP-18e; B 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 8.9
Limit of detection: 2.5 ng

OTHER SUBSTANCES

Extracted: dicloxacillin, oxacillin, penicillin V, penicillin G

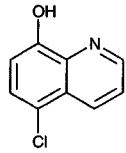
KEY WORDS

post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE

Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

Cloxyquin



Molecular formula: C_9H_6ClNO
Molecular weight: 179.61
CAS Registry No.: 130-16-5
Merck Index: 2483

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 100 μg/mL solution in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChroSpher 60 RP-Select-B
Mobile phase: MeOH:pH 2 trifluoroacetic acid buffer 40:60
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Retention time: 5.32

OTHER SUBSTANCES

Simultaneous: chlorphenesin, naftifine, sulbentine, tolnaftate

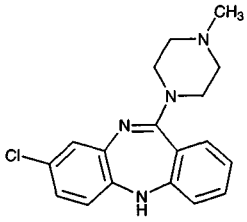
KEY WORDS

photodegradation; kinetics

REFERENCE

Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

Clozapine



Molecular formula: $C_{18}H_{19}ClN_4$
Molecular weight: 326.83
CAS Registry No.: 5786-21-0
Merck Index: 2484
Lednicer No.: 2 425, 4 212, 220

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 4 µg/mL amoxapine in 100 mM HCl + 400 µL 1 M NaOH + 6 mL ethyl acetate, shake for 15 min, centrifuge at 4000 g for 10 min. Transfer top organic layer to another tube and re-extract the analyte with 3 mL 50 mM HCl. Evaporate organic layer under nitrogen at 45°. Dissolve the residue in 120 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: Perisorb RP-8 (Upchurch)
Column: 100 × 4.6 3 µm Supelco C8-DB
Mobile phase: MeCN:MeOH:buffer 20:18:62 (Buffer was 50 mM monobasic sodium phosphate containing 2.5 mL/L triethylamine adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 30
Detector: UV 230

CHROMATOGRAM

Retention time: 6.5
Internal standard: amoxapine (14.3)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amitryptiline, atenolol, carbamazepine, cogentin, desipramine, desmethylsertraline, diazepam, doxepin, fluoxetine, haloperidol, imipramine, loxapine, medazepam, nortryptiline, oxazepam, paroxetine, phenytoin, propranolol, sertraline, thiothixene, trazadone, trifluoperazine, valproic acid, verapamil
Interfering: bupropion

KEY WORDS

serum; pharmacokinetics

REFERENCE

Hariharan,U.; Hariharan,M.; Naickar,J.S.; Tandon,R. Determination of clozapine and its two major metabolites in human serum by liquid chromatography using ultraviolet detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2409–2417.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 µL 660 nM IS in MeOH:water 5:95. Add to the SPE cartridge. Wash twice with 2 mL water. Acidify the SPE cartridge with 1 mL MeOH:250 mM HCl 10:90, wash with 500 µL MeCN, elute twice with 500 µL 10 mM acetic acid and twice with 500 µL 5 mM diethylamine in MeOH. Evaporate the eluate to dryness with a gentle stream of air at 37°. Reconstitute the residue with 100 µL mobile phase. Inject a 40 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm Select-B C8 (Merck)
Mobile phase: MeCN:MeOH:10 mM pH 3.7 dipotassium hydrogen phosphate 30:2:100
Flow rate: 1.5
Injection volume: 40
Detector: UV 220

CHROMATOGRAM
Retention time: 5
Internal standard: protriptyline (8.5)
Limit of detection: 15 nM
Limit of quantitation: 50 nM

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, clomipramine, chlorpromazine, chlorprothixene, citalopram, desipramine, diazepam, doxepin, fluoxetine, haloperidol, imipramine, levomepromazine, maprotiline, medazepam, mianserin, midazolam, nitrazepam, norclomipramine, nordoxepin, norfluoxetine, normaprotiline, nortrimipramine, nortryptiline, thioridazine, thiothixene, trazodone, trimipramine
Noninterfering: carbamazepine, carbamazepine-10-epoxide, carbamazepine-11-epoxide, clobazam, ethosuximide, flunitrazepam, 10-hydroxycarbazepine, norclobazam, oxazepam, oxcarbazepine, pentobarbital, phenobarbital, primidone, temazepam
Interfering: desmethylcitalopram

KEY WORDS
plasma; serum; SPE; pharmacokinetics

REFERENCE
Åkerman,K.A. Analysis of clozapine and norclozapine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *696*, 253–259.

SAMPLE
Matrix: blood
Sample preparation: Mix 25 μL 1.5 μg/mL IS, 50 μL serum, and 100 μL 1 M pH 9.0 borate buffer, add 1 mL chloroform, vortex for 1 min, centrifuge at 1100 g for 5 min. Evaporate the organic layer to dryness at 40° under nitrogen, resuspend in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5 μm Symmetry C18 Waters
Mobile phase: MeCN:MeOH:buffer 20:10:70 (Buffer was 28.6 mM sodium acetate, pH adjusted to 2.6 with 40% phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4.5
Internal standard: α-hydroxymidazolam (Hoffman-La Roche)(8)
Limit of detection: 2.5 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: N-desmethylclozapine metabolite

KEY WORDS
pharmacokinetics; rat; serum; validation

REFERENCE
Ma,F.; Lau,C.E. Determination of clozapine and its metabolite, N-desmethylclozapine, in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1998**, *712*, 193–198.

SAMPLE
Matrix: blood
Sample preparation: Mix 400 μL plasma with 50 μL protein releasing reagent. Dialyze 100 μL of this mixture, using a cellulose acetate (Gilson Cuprophane, molecular weight cut-off 15000) membrane, against 4 mL 2 mM pH 4.0 ammonium acetate buffer pumped through a 175 μL channel at 0.47 mL/min. The dialysate flowed through column A to waste. When dialysis is complete elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. Wash the donor channel of the dialysis apparatus with 5 mL 1 mM dodecylethyldimethyl ammonium bromide and the acceptor channel with 5 mL 2 mM pH 4.0 ammonium acetate buffer. (The protein-releasing reagent was aqueous solution of 1 M HCl, 20 mM dodecylethyldimethyl ammonium bromide (Fluka), and 50% (v/v) glycerol.)

HPLC VARIABLES
Column: A 10 × 2 40 μm BondElut C18 (Varian); B 100 × 4.6 5 μm Brownlee CN (Applied Biosystems)
Mobile phase: MeCN:50 mM pH 3.2 ammonium acetate buffer 22:78
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 4.1
Limit of detection: 50 nM
Limit of quantitation: 250 nM

OTHER SUBSTANCES
Extracted: N-desmethylclozapine

KEY WORDS
plasma; dialysis

REFERENCE
Johansen,K.; Krogh,M.; Rasmussen,K.E. Automated on-line dialysis, trace enrichment and high-performance liquid chromatography. Inhibition of interaction with the dialysis membrane and disruption of protein binding in the determination of clozapine in human plasma, *J.Chromatogr.B*, **1997**, *690*, 223–231.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma or 1 mL diluted red blood cells (red blood cells: water 50:50) with 300 ng loxapine, mix briefly by manual agitation. Add 6 mL ethyl acetate, vortex for 2 min, centrifuge at 2000 g for 5 min. Remove the organic layer, add 250 μL 100 mM HCl, vortex for 2 min, centrifuge at 2000 g for 5 min. Remove a 200 μL volume of the acid layer, evaporate to dryness at 40°. Reconstitute the residue in 200 μL mobile phase and inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 250 × 4.6 5 μm Kromasil Ultrabase C18
Mobile phase: MeCN:buffer 48:52 (Buffer was 716 mg disodium hydrogen phosphate and 2 g cetrimide in 1 L water, adjusted to pH 7.0 with phosphoric acid.)
Column temperature: 50

Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 11.0
Internal standard: loxapine (20.0)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clonazepam, clorazepate, droperidol
Noninterfering: clomipramine, diazepam, hydroxyzine

KEY WORDS
liquid-liquid extraction; plasma; red blood cells; pharmacokinetics

REFERENCE
Guitton,C.; Kinowski,J.-M.; Aznar,R.; Bressolle,F. Determination of clozapine and its major metabolites in human plasma and red blood cells by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *690*, 211–222.

SAMPLE
Matrix: blood
Sample preparation: Add 40 μL 10 μg/mL loxapine to 1 mL plasma, add 200 μL 330 mM NaOH, vortex, add 6 mL hexane:isoamyl alcohol 98.5:1.5, shake for 30 min, centrifuge at 4000 rpm for 5 min. Collect the organic layer, add 150 μL 100 mM HCl, vortex for 1 min. Collect the acidic aqueous phase and inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 C8 (Bischoff, Germany)
Column: 125 × 4.6 5 μm Ecotube Nucleosil C8 (Bischoff, Germany)
Mobile phase: MeCN:Pic B5:water:diethylamine 37:2.5:63:0.04 (Pic B5 is a mixture of water, MeOH, 1-pentanesulfonic acid, and acetic acid and is available from Waters.)
Column temperature: 56
Flow rate: 1.7
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 8.7
Internal standard: loxapine (12.7)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: alprazolam, bromazepam, chlordiazepoxide, clorazepate, diazepam, flunitrazepam, haloperidol, lorazepam, nitrazepam, oxazepam, paroxetine, prazepam, temazepam, triazolam

KEY WORDS
plasma

REFERENCE
Edno,L.; Combourieu,I.; Cazenave,M.; Tignol,J. Assay for quantitation of clozapine and its metabolite N-desmethylclozapine in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 311–318.

SAMPLE
Matrix: solutions

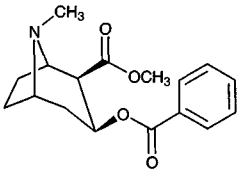
HPLC VARIABLES
Column: 100 × 4.6 Microsorb CN
Mobile phase: MeCN:MeOH:50 mM pH 6.5 sodium phosphate buffer 5:28:67
Column temperature: 37
Flow rate: 1.5

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: olanzapine, paroxetine
Also analyzed: haloperidol, risperidone
Interfering: imipramine

REFERENCE
Prieto,I.V.; Hoffman,D.W. HPLC monitoring of olanzapine (Abstract 131), *Ther.Drug Monit.*, **1997**, *19*, 580–580.

Cocaine



Molecular formula: $C_{17}H_{21}NO_4$
Molecular weight: 303.36
CAS Registry No.: 50-36-2, 53-21-4 (HCl)
Merck Index: 2517

SAMPLE

Matrix: blood
Sample preparation: 100 µL Plasma + 50 µL 5 µg/mL IS + 500 µL pH 6 phosphate buffer + 10 mL chloroform, shake for 10 min (Caution! Chloroform is a carcinogen!). Evaporate the chloroform layer under a stream of nitrogen. Reconstitute the residue in 75 µL mobile phase. Inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 µm Supelcosil ABZ+
Mobile phase: MeCN:50 mM ammonium phosphate 9:91
Flow rate: 0.6
Injection volume: 20
Detector: UV 233

CHROMATOGRAM

Internal standard: lidocaine
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Hedaya,M.A.; Pan,W.-J. Cocaine and alcohol interactions in naive and alcohol-pretreated rats, *Drug Metab.Dispos.*, **1996**, *24*, 807–812.

SAMPLE

Matrix: blood
Sample preparation: Add 1 ml buffer to 1 mL serum, vortex for 5 s. Add 5 ml hexane and shake on an oscillating shaker for 3 min. Centrifuge at 1200 g for 3 min and freeze lower aqueous layer in acetone/dry ice mixture. Evaporate hexane layer to dryness under a stream of nitrogen. Reconstitute with 200 µL mobile phase. Inject an aliquot. (Keep the sample in the autosampler at 2°. Prepare the buffer by mixing 24 mL 100 mM sodium carbonate with 176 mL 100 mM sodium hydrogen carbonate.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:pH 7.4 phosphate buffer 38:62
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Retention time: 9.3
Internal standard: lidocaine

OTHER SUBSTANCES
Extracted: cocaethylene, cocaine, norcocaine

KEY WORDS
dog; serum; lidocaine is IS

REFERENCE
Williams,C.L.; Laizure,S.C.; Parker,R.B.; Lima,J.J. Quantitation of cocaine and cocaethylene in canine serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 271–276.

SAMPLE
Matrix: blood
Sample preparation: Mix 50 μL 8 μ g/mL bupivacaine in water with 500 μL pH 6.0 buffer and 100 μL plasma sample, add 10 mL chloroform, shake mechanically for 10 min, centrifuge at 873 g for 10 min, evaporate the organic layer under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 35 μL aliquot. (Prepare pH 6.0 buffer by mixing 67 mM KH_2PO_4 with 67 mM Na_2HPO_4 in an 87.7:12.3 ratio.)

HPLC VARIABLES
Column: 250 × 2.1 5μm Supelcosil ABZ+plus deactivated reversed-phase
Mobile phase: MeOH:MeCN:50 mM pH 4.5 monobasic ammonium phosphate 5:7:63
Flow rate: 0.4
Injection volume: 35
Detector: UV 235

CHROMATOGRAM
Retention time: 12.38
Internal standard: bupivacaine (26.90)
Limit of detection: 24 ng/mL
Limit of quantitation: 81 ng/mL

OTHER SUBSTANCES
Extracted: benzoylecgonine, norcocaine, cocaethylene, metabolites
Simultaneous: ascorbic acid, morphine, oxymorphone, noroxymorphone, norhydromorphone, norcodeine, codeine, nalorphine, procaine, acetaminophen, oxycodone, hydrocodone, caffeine, ethylmorphine, lidocaine, benzoylnorecgonine, ketamine, acepromazine, salicylic acid, benzoic acid, thebaine, cocaine propyl ester, benzocaine, tetracaine, pentobarbital

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Pan,W.-J.; Hedaya,M.A. Sensitive and specific high-performance liquid chromatographic assay with ultraviolet detection for the determination of cocaine and its metabolites in rat plasma, *J.Chromatogr.B*, **1997**, *703*, 129–138.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 10 μg/mL lidocaine in MeOH to 1 mL serum, vortex for 5 s. Add 1 ml buffer, vortex for 5 s. Add 5 ml hexane and shake on an oscillating shaker for 3 min. Centrifuge at 1200 g for 3 min and freeze lower aqueous layer in acetone/dry ice mixture. Evaporate hexane layer to dryness under a stream of nitrogen. Reconstitute with 200 μL mobile phase. Inject an aliquot. (Keep the sample in the autosampler at 2° Prepare the buffer by mixing 24 mL 100 mM sodium carbonate with 176 mL 100 mM sodium hydrogen carbonate.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: MeCN:pH 7.4 phosphate buffer 38:62
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 16.8
Internal standard: lidocaine (9.3)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, cocaethylene, norcocaine

KEY WORDS
dog; serum; pharmacokinetics

REFERENCE
Williams,C.L.; Laizure,S.C.; Parker,R.B.; Lima,J.J. Quantitation of cocaine and cocaethylene in canine serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 271–276.

SAMPLE
Matrix: blood
Sample preparation: Add 25 μL 4 μg/mL IS to 50 μL serum, add 100 μL 1 M pH 9.0 borate buffer, mix well, add 1 mL EtOH:chloroform 12.5:87.5, vortex for 1 min, centrifuge at 1100 g for 5 min. Evaporate the organic layer to dryness under nitrogen at 40°. Resuspend the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:buffer 10:12.5:77.5 containing 129 μM tetrabutylammonium phosphate (Buffer was 25.8 mM sodium acetate adjusted to pH 2.2 with 40% phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: k' 4.37
Internal standard: 3-isobutyl-1-methylxanthine (k' 7.85)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, benzoylecgonine, benzoylnorecgonine, norcocaine
Noninterfering: barbital, caffeine, cocaethylene, flurazepam, hexobarbital, lidocaine, mazindol, norcocaethylene

KEY WORDS
pharmacokinetics; rat; serum

REFERENCE
Ma,F.; Zhang,J.; Lau,C.E. Determination of cocaine and its metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1997**, *693*, 307–312.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 5.8
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS
serum

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 25 μg/mL lidocaine in 4 mM HCl, mix, add 100 μL 1 M pH 9.0 borate buffer, add 1 mL chloroform:EtOH 82.5:17.5, mix, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:buffer 12:16:72 (Buffer was 31 mM sodium acetate adjusted to pH 5.1 with 40% phosphoric acid containing 0.15 mM tetrabutylammonium phosphate.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 15
Internal standard: lidocaine (10)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, metabolites
Simultaneous: barbital, phenobarbital, flumazepil, mazindol, hexobarbital, nicotine, procaine, cotinine
Noninterfering: amphetamine, desipramine, tetracaine, methadone, reserpine, buspirone, diazepam, haloperidol, chlordiazepoxide, oxazepam, midazolam, clonazepam, chlorpromazine, pentobarbital

KEY WORDS
serum; rat

REFERENCE
Lau,C.E.; Ma,F.; Falk,J.L. Simultaneous determination of cocaine and its metabolites with caffeine in rat serum microsamples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *532*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 40 μg benzoctamine, extract with dichloromethane: isopropanol 80:20

HPLC VARIABLES
Guard column: 30 × 3 7 μm Separon SGX CN (Tessek)
Column: 150 × 3 7 μm Separon SGX CN (Tessek)
Mobile phase: MeOH:buffer 15:85 (Buffer was 100 mM phosphate containing 1 mL/L triethylamine, pH adjusted to 3.5 with phosphoric acid.)
Flow rate: 0.7
Detector: UV 233

CHROMATOGRAM
Retention time: 10
Internal standard: benzoctamine (15)
Limit of detection: 200 ng/mL (urine), 50 ng/mL (serum)

OTHER SUBSTANCES
Extracted: benzoylecgonine

KEY WORDS
serum

REFERENCE
Balíková,M.; Vecerková,J. High-performance liquid chromatographic confirmation of cocaine and benzoylecgonine in biological samples using photodiode-array detection after toxicological screening, *J.Chromatogr.B*, **1994**, *656*, 267–273.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 24

Limit of detection: 0.20 ppm

OTHER SUBSTANCES

Extracted: buprenorphine, caffeine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS

whole blood

REFERENCE

Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Clean Screen SPE cartridge (Worldwide Monitoring) with two 2 mL portions of MeOH, with 3 mL water, and with 3 mL 10 mM pH 3.0 phosphate buffer. 1 mL Serum + bupivacaine + 500 μL 10 mM pH 3.0 phosphate buffer, mix, add to the SPE cartridge, air dry for 30 s, wash with 3 mL phosphate buffer, wash with 3 mL 100 mM HCl, wash with 3 mL MeOH, elute with 2 mL chloroform:isopropanol: ammonium hydroxide 22:20.5:2.5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 SemiPermeable Surface (SPS) C8 (Regis)
Mobile phase: THF:2.5 mM potassium phosphate buffer 3.25:96.75 containing 0.0025% triethylamine, final pH adjusted to 2.7-2.8 with 85% orthophosphoric acid
Flow rate: 0.5
Detector: UV 235

CHROMATOGRAM

Retention time: 15.5
Internal standard: bupivacaine (24)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: acepromazine, atropine, benzoylecgonine, benzoylnorecgonine, ketamine, norcocaine
Noninterfering: benzethonium chloride, benzyl alcohol

KEY WORDS

SPE; serum

REFERENCE

Muztar,J.; Chari,G.; Bhat,R.; Ramaro,S.; Vidyasagar,D. A high-performance liquid chromatographic procedure for the separation of cocaine and some of its metabolites from acepromazine, ketamine, and atropine from serum, *J.Liq.Chromatogr.*, **1995**, *18*, 2635–2645.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min,

inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 4.75
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 300 mg Bond Elut Certify SPE cartridge with 6 mL MeOH, 3 mL water, and 5 mL 10 mM pH 2.0 NaH_2PO_4. 1 mL Plasma + 100 μL 20 μg/mL tropacocaine in water + 3 mL 10 mM pH 2.0 NaH_2PO_4, vortex briefly, add to the SPE cartridge at 0.5 mL/min, dry under vacuum for 1-2 min, wash with 3 mL water at 1 mL/min, dry under vacuum for 3 min, wash with 3 mL 100 mM HCl, dry under vacuum for 3 min, wash rapidly with 6 mL MeOH, dry under vacuum for 5 min, elute with 6 mL dichloromethane:isopropanol:ammonium hydroxide 80:20:2 without vacuum. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 2 mL dichloromethane:isopropanol 80:20. Remove a 1 mL aliquot and evaporate it to dryness under a stream of nitrogen, reconstitute with 300 μL mobile phase, mix for 30 s, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee C8

Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:buffer:triethylamine 18:81.7:0.3 (Buffer was 50 mM citric acid:100 mM Na_2HPO_4 80:20, pH 3.0.)

Flow rate: 1.5

Injection volume: 100

Detector: UV 235

CHROMATOGRAM

Retention time: 17.6

Internal standard: tropacocaine (12.8)

Limit of detection: 75 ng/mL

Limit of quantitation: 220 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, benzoylecgonine, norcocaine

KEY WORDS

rat; plasma; SPE

REFERENCE

Virag,L.; Mets,B.; Jamdar,S. Determination of cocaine, norcocaine, benzoylecgonine and ecgonine methyl ester in rat plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *681*, 263–269.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 220

CHROMATOGRAM
Retention time: 8.97
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, hair
Sample preparation: Hair. Wash 20-200 mg hair twice with 20 mL aliquots of 0.3% Tween 20 in water, rinse thoroughly with water, dry at 37°, add 2 mL 250 mM HCl, heat at 45° overnight, neutralize with 1 M NaOH, extract twice with Isolute Confirm HCX (from IST, U.K.), combine the organic layers and evaporate them to dryness, reconstitute with 500 μL 50 mM pH 5.2 NaH_2PO_4, inject a 100-200 μL aliquot. Plasma. 200 μL Plasma + 150 μL 100 mM pH 8.9 Na_2HPO_4 + 5 mL chloroform:isopropanol 90:10, vortex for 2 min, centrifuge at 700 g for 10 min. Evaporate 4 mL of the organic phase, reconstitute the residue in 500 μL pH 5.2 50 mM NaH_2PO_4, inject a 100-200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Bio-Gel PRP 70-5 poly(styrene-divinylbenzene) (Bio-Rad)
Mobile phase: MeOH:THF:100 mM pH 3 potassium phosphate 25:5:70
Flow rate: 0.5
Injection volume: 100-200
Detector: F ex 230 em 315

CHROMATOGRAM
Retention time: 20
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, amitriptyline, amobarbital, amphetamine, aprobarbital, atropine, barbital, benzoylecgonine, benztropine, butabarbital, caffeine, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, chlorprothixene, cimetidine, codeine, dextromethorphan, diazepam, dihydrocodeine, diphenhydramine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, ethinamate, ethylmorphine, flurazepam, glutethimide, hydrocodone, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, morphine, naloxone, nicotine, nordiazepam, nortriptyline, orphenadrine, oxycodone, papaverine, pentazocine, pentobarbital, phenacetin, phencyclidine, phenmetrazine, phenobarbital, phenolphthalein, phentermine, phenylpropanolamine, phenytoin, phetidine, prazepam, procainamide, procaine, propoxyphene, propranolol, protriptyline, pseudoephedrine, pyrilamine, quinine,

salicylamide, secobarbital, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, trimethoprim, trimetobenzamide

KEY WORDS
plasma

REFERENCE
Tagliaro,F.; Antonioli,C.; De Battisti,Z.; Ghielmi,S.; Marigo,M. Reversed-phase high-performance liquid chromatographic determination of cocaine in plasma and human hair with direct fluorimetric detection, *J.Chromatogr.A*, **1994**, *674*, 207–215.

SAMPLE
Matrix: blood, saliva, tissue, urine

Sample preparation: Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5 NaH_2PO_4 containing 0.5% NaF. Add 500 μL brain homogenate or 500 μL plasma, saliva, or urine containing 15 μL saturated NaF solution to 75 μL 150 μg/mL IS, add 50 μL 50% perchloric acid, mix vigorously for 10 s, let stand at room temperature for 10 min, add 1 mL water, mix briefly, centrifuge at 10° at 2500 (?) for 30 min. Remove the supernatant and add it to 750 μL saturated sodium carbonate solution, mix briefly, add 7.5 mL pentane:chloroform 95:5, rock gently for 10 min, centrifuge in a desk-top centrifuge for 2 min, freeze in dry ice/acetone for 2 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix vigorously for 10 s, centrifuge in a desk-top centrifuge for 1-2 min, freeze in dry ice/acetone for 3-5 min, discard the organic layer. Allow the aqueous layer to thaw, remove any trace of organic solvent with a stream of nitrogen, inject a 75 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee RP-8

Column: 250 × 4.6 5 μm Zorbax RX-C18

Mobile phase: MeCN:buffer 18:82 (Buffer was 100 mM K_2HPO_4 containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)

Flow rate: 2

Injection volume: 75

Detector: UV 235

CHROMATOGRAM
Retention time: 6.5

Internal standard: 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

Limit of detection: 5 ng/g (brain), 5 ng/mL (plasma)

Limit of quantitation: 25 ng/g (brain), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: chlordiazepoxide, clozapine, gepirone, methylphenidate, pentazocine, pseudococaine

Simultaneous: acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, benperidol, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hydergine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaquaalude, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylecgonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428

Noninterfering: amitriptyline, benztropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobam-

ate, methadone, methamphetamine, nicotine, norfluoxetine, nortriptyline, PCP, phenothiazine, pseudoephedrine

KEY WORDS
rat; cow; plasma; brain

REFERENCE
Bonate,P.L.; Davis,C.M.; Silverman,P.B.; Swann,A. Determination of cocaine in biological matrices using reversed phase HPLC: Application to plasma and brain tissue, *J.Liq.Chromatogr.*, **1995**, *18*, 3473–3494.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Condition a Bond-Elut Certify SPE cartridge with 2 mL MeOH and 2 mL pH 6 phosphate buffer. Mix 2.5 mL urine and 2.5 mL water, add to the SPE cartridge, wash with 3 mL water, wash with 3 mL 100 mM HCl, wash with 9 mL MeOH, wash with 3 mL ammonia, dry column under vacuum for 5 min, elute with 2 mL chloroform:isopropanol 80:20. Evaporate 1.5 mL of the eluate to dryness under a stream of nitrogen at 65°, reconstitute with 500 μL 1 μg/mL methaqualone in mobile phase, inject a 25 μL aliquot. Plasma. Condition a Bond-Elut Certify SPE cartridge with 2 mL MeOH and 2 mL pH 6 phosphate buffer. Mix 1 mL plasma and 2 mL water, add to the SPE cartridge, wash with 3 mL water, wash with 3 mL 100 mM HCl, wash with 9 mL MeOH, wash with 3 mL ammonia, dry column under vacuum for 5 min, elute with 2 mL chloroform:isopropanol 80:20. Evaporate 1.5 mL of the eluate to dryness under a stream of nitrogen at 65°, reconstitute with 100 μL 1 μg/mL methaqualone in mobile phase, inject a 25 μL aliquot. (Prepare pH 6 phosphate buffer by adding 55 mL 100 mM Na_2HPO_4 to 445 mL 100 mM KH_2PO_4.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher RP18
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeOH:pH 7 phosphate buffer 70:30 (Prepare buffer by adding 320 mL 20 mM K_2HPO_4 to 680 mL 20 mM KH_2PO_4.)
Flow rate: From 0.4 to 0.7 over 6 min, to 1.0 over 2 min, to 0.7 over 2 min, to 0.6 over 1 min, to 0.4 over 1 min
Injection volume: 25
Detector: UV 235

CHROMATOGRAM
Retention time: 8.8
Internal standard: methaqualone (5.8)
Limit of detection: 5 ng/mL (urine), 12.5 ng/mL (plasma)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: benzoylecgonine
Simultaneous: acetaminophen, amitriptyline, atropine, benzocaine, caffeine, codeine, diazepam, flunitrazepam, imipramine, ketazolam, lidocaine, meperidine, methadone, midazolam, morphine, nordiazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, procaine, propoxyphene, secobarbital, tetracaine, tetrazepam
Noninterfering: amphetamine, clomipramine, haloperidol, maprotiline, mianserin, thioridazine

KEY WORDS
SPE

REFERENCE
Fernandez,P.; Lafuente,N.; Bermejo,A.M.; Lopez-Rivadulla,M.; Cruz,A. HPLC determination of cocaine and benzoylecgonine in plasma and urine from drug abusers, *J.Anal.Toxicol.*, **1996**, *20*, 224–228.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 10 μL 100 μg/mL bupivacaine + 1 mL buffer + 8 mL chloroform:isopropanol 60:40, shake for 10 min, centrifuge. Remove the organic layer and add it to 2 mL 100 mM sulfuric acid, shake for 10 min, centrifuge. Remove the aqueous layer and add it to 2 mL buffer and 10 mL chloroform:isopropanol 60:40, shake for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot. (Buffer was saturated sodium carbonate:saturated sodium bicarbonate 50:50.)

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM ammonium acetate 30:30:40
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Internal standard: bupivacaine (11)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: benzoylecgonine
Interfering: lidocaine

KEY WORDS
whole blood; plasma

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or 500 μL urine + 50 μL 1 μg/mL tropacocaine in water + 200 μL 500 mM pH 9.1 carbonate buffer + 2 mL ethyl acetate, shake for 10 min, centrifuge at 1200 g for 3 min. Remove the organic phase and add it to 150 μL 50 mM HCl, shake for 10 min, centrifuge at 1200 g for 3 min, evaporate the aqueous phase with a centrifugal evaporator, reconstitute the residue with 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C8
Mobile phase: MeCN:buffer 24:76 (Buffer was 50 mM pH 6.0 KH_2PO_4 containing 100 mM pentanesulfonic acid.)
Flow rate: 1.3
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 8.0
Internal standard: tropacocaine (6.3)
Limit of quantitation: 5 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites, cocaethylene, norcocaethylene, norcocaine

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Sukbuntherng,J.; Walters,A.; Chow,H.-H.; Mayersohn,M. Quantitative determination of cocaine, cocaethylene (ethylcocaine), and metabolites in plasma and urine by high-performance liquid chromatography, *J.Pharm.Sci.*, **1995**, *84*, 799–804.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a Bond Elut C8 SPE cartridge with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Plasma or serum + 100 μL MeOH + 200 μL MeCN + 100 μL buffer, vortex for 1 min, centrifuge at 4000 rpm for 15 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 5 μg/mL nalorphine in MeOH (?), inject an aliquot. Urine. Condition a Bond Elut C8 SPE cartridge with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Urine + 100 μL MeOH + 200 μL MeCN + 500 μL buffer, vortex for 1 min, centrifuge at 2000 rpm for 5 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 5 μg/mL nalorphine in MeOH (?), inject an aliquot. (Buffer was 250 mL 25 mM sodium borate and 18 mL 100 mM NaOH, pH 9.2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:MeOH:1.2% ammonium acetate 15:40:45
Flow rate: 0.8
Detector: UV 239

CHROMATOGRAM
Retention time: 14.55
Internal standard: nalorphine (9.72)
Limit of quantitation: 83 ng/mL (urine), 100 ng/mL (plasma, serum)

OTHER SUBSTANCES
Extracted: benzoylecgonine

KEY WORDS
SPE; plasma; serum

REFERENCE
Theodoridis,G.; Papadoyannis,I.; Tsoukali-Papadopoulou,H.; Vasilikiotis,G. A comparative study of different solid phase extraction procedures for the analysis of alkaloids of forensic interest in biological fluids by RP-HPLC/Diode array, *J.Liq.Chromatogr.*, **1995**, *18*, 1973–1975.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.92

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 100 × 5 10 μm Radial-Pak C18 radial compression (Waters)
Mobile phase: MeOH:100 mM NaCl:butylamine 50:50:0.7, adjusted to pH 3 with sulfuric acid
Flow rate: 1
Injection volume: 200
Detector: E, Bioanalytical systems LC4B, MF 1000 dual glassy carbon working electrode + 1.1 V and +0.75 V, MF 1018 stainless steel auxiliary electrode, MF 2020 Ag/AgCl reference electrode following post-column reaction. The column flowed through a knitted 9.1 m × 0.5 mm ID PTFE coil irradiated at 254 nm by a Photronix medium-pressure mercury lamp (cooled with ice-water) to the detector.

CHROMATOGRAM
Retention time: k' 0.53
Limit of detection: 500 ppb

OTHER SUBSTANCES
Simultaneous: amylocaine, ascorbic acid, caffeine, chloroprocaine, lidocaine, niacinamide, procaine
Noninterfering: inositol, lactose, mannitol

KEY WORDS
post-column reaction; post-column photochemical derivatization

REFERENCE
Selavka,C.M.; Krull,I.S.; Lurie,I.S. An improved method for the rapid screening of illicit cocaine preparations using high performance liquid chromatography with electrochemical detection, *Forensic Sci.Int.*, **1986**, *31*, 103–117.

SAMPLE
Matrix: bulk
Sample preparation: Weigh out 50 mg of the hydrochloride, dissolve in 2 mL 24 μg/mL meconin and 60 μg/mL n-butyrophenone in MeCN:buffer 20:80, vortex, inject a 150 μL aliquot. (Buffer was 40 mL phosphoric acid and 120 mL 2 mM NaOH in 3480 mL water containing 20 mM sodium dodecyl sulfate, pH 2.0.)

HPLC VARIABLES
Column: 125 × 4.6 HS-5 C18 (Perkin-Elmer)
Mobile phase: Gradient. MeCN:THF:buffer 21.9:1.7:76.4 to MeCN:buffer 44:56 over 12 min (concave gradient), to MeOH:buffer 80:20 over 13 min (linear), maintain at MeOH:buffer 80:20 for 8 min, return to initial conditions over 5 min. (Buffer was 40 mL phosphoric acid and 120 mL 2 mM NaOH in 3480 mL water containing 20 mM sodium dodecyl sulfate, pH 2.0.)
Flow rate: 1.5
Injection volume: 150
Detector: UV 215 and 277

CHROMATOGRAM
Retention time: 15
Internal standard: meconin (2.5), n-butyrophenone (11.5)

OTHER SUBSTANCES
Simultaneous: impurities, benzoylecgonine

REFERENCE
Lurie,I.S.; Moore,J.M.; Cooper,D.A.; Kram,T.C. Analysis of manufacturing by-products and impurities in illicit cocaine via high-performance liquid chromatography and photodiode array detection, *J.Chromatogr.*, **1987**, *405*, 273–281.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 26.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 5 μL aliquot of dialysate.

HPLC VARIABLES
Column: 100 × 1 3 μm SepStik (BioAnalytical Systems, West Lafayette, IN)
Mobile phase: THF:50 mM NaH_2PO_4 24:76 containing 0.2 mM disodium EDTA, 3.7 mM 1-decanesulfonic acid, and 4.9 mM triethylamine, pH 5.5
Flow rate: 0.023
Injection volume: 5
Detector: E, (EG & G Princeton Applied, USA), Model MP 1304, glassy carbon electrode, first at +700 mV, second at 0 mV, Ag/AgCl reference electrode (BioAnalytical Systems, Model RE4); UV 225

CHROMATOGRAM
Retention time: 25
Limit of quantitation: 100 nM

OTHER SUBSTANCES
Simultaneous: dopamine

KEY WORDS
rat; brain; microdialysis; pharmacokinetics

REFERENCE
Parsons,L.H.; Kerr,T.M.; Weiss,F. Simple microbore high-performance liquid chromatographic method for the determination of dopamine and cocaine from a single in vivo brain microdialysis sample, *J.Chromatogr.B*, **1998**, *709*, 35–45.

SAMPLE
Matrix: leaves
Sample preparation: Reflux 0.05-4 g air-dried coca leaves in 30-100 mL 95% EtOH for 15 min, filter (Whatman No. 4 paper), inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm C8 (R.E.Gourley)
Mobile phase: MeCN:1% triethylamine pH 4 40:60
Flow rate: 1.2
Injection volume: 5
Detector: UV 240

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 5 ng

REFERENCE
Glass,R.L.; Johnson,E.L. Comparison of high performance liquid chromatographic and gas chromatographic analyses of cocaine in coca leaves, *J.Liq.Chromatogr.*, **1993**, *16*, 3543–3555.

SAMPLE
Matrix: meconium
Sample preparation: Condition a 1 mL 100 mg Bond Elut strong cation exchange SPE cartridge with 2 mL MeOH, 1 mL water, and 1 mL 250 mM phosphate buffer. 0.5 g Meconium + 2 mL MeOH, vortex for 1 min, centrifuge at 450 g for 5 min. Remove the supernatant and add it to 1 mL 25 mM pH 3 potassium phosphate buffer, add to SPE cartridge, air dry for 30 s, wash with 1 mL phosphate buffer, wash with 100 mM acetic acid, air dry for 30 s, elute with 2 mL 3% ammonia in MeOH, evaporate eluate to dryness under a stream of nitrogen, reconstitute in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4.5 C18 Guard Pak (Waters)
Column: 250 × 4.5 5 μm Spherisorb ODS
Mobile phase: MeCN:25 mM potassium phosphate buffer 15:85 containing 25 mL/L diethylamine, final pH adjusted to 2.9 with concentrated orthophosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 230, 255, 270

CHROMATOGRAM
Retention time: 11.0

OTHER SUBSTANCES
Extracted: benzoylecgonine

KEY WORDS
SPE

REFERENCE
Browne,S.P.; Tebbett,I.R.; Moore,C.M.; Dusick,A.; Covert,R.; Yee,G.T. Analysis of meconium for cocaine in neonates, *J.Chromatogr.*, **1992**, *575*, 158–161.

SAMPLE
Matrix: meconium
Sample preparation: Condition a 200 mg Clean-Screen DAU SPE cartridge (Worldwide Monitoring) with two 3 mL portions of MeOH, 3 mL water, and 3 mL pH 3 buffer. 0.5 g Meconium + 2 mL MeOH, vortex, centrifuge. Remove the supernatant and add it to 3 mL pH 3 phosphate buffer and 20 μL 100 ng/mL bupivacaine, mix, add to the SPE cartridge, air dry, wash with 3 mL buffer, wash with 3 mL 100 mM HCl, wash with 3 mL MeOH, elute with three 1 mL portions of chloroform:isopropanol:ammonium hydroxide 78:20:2. Evaporate the eluate to dryness without heating, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: Guard-Pak C18 (Waters)
Column: 300 × 3.9 μBondapak C18 ODS
Mobile phase: MeCN:butylamine:25 mM KH_2PO_4 125:12.5:500, pH adjusted to 2.9 with 85% phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 230, UV 255, UV 275

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE

Browne,S.; Moore,C.; Negrusz,A.; Tebbett,I.; Covert,R.; Dusick,A. Detection of cocaine, norcocaine, and cocaethylene in the meconium of premature neonates, *J.Forensic Sci.*, **1994**, *39*, 1515–1519.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimetho-

benzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH or water to 0.1%.

HPLC VARIABLES

Column: two 250 mm β-cyclodextrin bonded phase columns in series (Advanced Separation Technologies)
Mobile phase: MeCN:1% pH 4.1 aqueous triethylammonium acetate 4:96
Flow rate: 0.5
Injection volume: 1
Detector: UV

CHROMATOGRAM

Retention time: 87 (d-isomer), 91 (l-isomer)

OTHER SUBSTANCES

Simultaneous: scopolamine
Interfering: atropine

KEY WORDS

chiral

REFERENCE

Armstrong,D.W.; Han,S.M.; Han,Y.I. Separation of optical isomers of scopolamine, cocaine, homatropine, and atropine, *Anal.Biochem.*, **1987**, *167*, 261–264.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 10 μm PRP-1 (Hamilton)
Mobile phase: Gradient. MeCN:20 mM ammonium hydroxide from 15:85 to 100:0 over 17 min
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: codeine, methadone, reserpine, thebaine, yohimbine

REFERENCE

Keystone Scientific Catalog, 1993-4, p. 22.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 3.905

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993,** *12(3)*, 18–21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, me-

phobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.00 (A), 4.99 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac,

labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Condition a Varian 1 mL SCX SPE ion exchange cartridge with 2 mL MeOH, 1 mL water, and 1 mL 250 mM pH 3.0 phosphate buffer. Dilute urine specimens 1:1 with 250 mM phosphate buffer, add 500 ng/mL IS, add to SPE cartridge, dry under vacuum for 30 s, wash with 1 mL phosphate buffer, wash with 500 μL 100 mM acetic acid, wash with 1 mL MeOH. Dry column again for 30 s, elute with 1.5 mL 3% ammonium hydroxide in MeOH. Evaporate the eluate to dryness under nitrogen, reconstitute with 100 μL MeOH, evaporate half of this solution to dryness under a stream of nitrogen at 60-70°, reconstitute in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Novapak Guard pak precolumn
Column: 250 × 4.6 10 μm Lichrosorb RP-18
Mobile phase: MeCN:25 mM KH_2PO_4 buffer: butylamine (18:81:1, v/v/v), adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 10.27
Internal standard: bupivacaine (15.55)
Limit of detection: 1 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, benzoylecgonine, cocaethylene, norcocaine

KEY WORDS
SPE; comparison with GC-MS

REFERENCE

Phillips,D.L.; Tebbett,I.R.; Bertholf,R.L. Comparison of HPLC and GC-MS for measurement of cocaine and metabolites in human urine, *J.Anal.Toxicol.*, **1996**, *20*, 305–308.

SAMPLE

Matrix: urine

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)

Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.

Flow rate: 1

Injection volume: 50

Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 304

CHROMATOGRAM

Retention time: 21

Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES

Extracted: 6-acetylmorphine, amphetamine, benzoylecgonine, ephedrine methamphetamine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS

SPE

REFERENCE

Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE

Matrix: urine

Sample preparation: Condition a 3 mL Bond Elut Certify C8/SCX SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 6 phosphate buffer (all flow rates are 1 mL/min). Mix 2 mL urine, 3 mL water, 2 mL 100 mM pH 6 phosphate buffer, and 100 μL internal standard solution (containing 13 μg/mL of both 2′-methylbenzoylecgonine and 2′-methylcocaine), adjust pH to 4.0-6.0 if necessary, add to the SPE cartridge, wash with 3 mL HPLC water, 3 mL 100 mM HCl, three 3 mL portions of MeOH and three 3 mL portions of MeCN. Elute with 2 mL dichloromethane:2-propanol:25% ammonium hydroxide 80:20:2. Evaporate the eluate at 35° under a stream of nitrogen.

HPLC VARIABLES

Guard column: 7.5 × 4.6 5 μm Hypersil BDS C18

Column: 150 × 4.6 5 μm Hypersil BDS C18

Mobile phase: Gradient. A was MeCN:MeOH:water 10:10:80 containing 45 mM ammonium acetate. B was MeCN:MeOH:water 40:40:20. A:B from 100:0 to 47.2:52.8 in 19 min, maintain at 47.2:57.8 for 2 min, to 100:0 in 1 min, maintain at 100:0 for 5 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM
Retention time: 13.7
Internal standard: 2′-methylbenzoylecgonine (9.7), 2′-methylcocaine (17.7) [Synthesis described in paper.]
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, acetylcodeine, alprenolol, amphetamine, apomorphine, bromazepam, caffeine, citalopram, codeine, dihydrocodeine, hydrocodone, hydromorphone, methamphetamine, methylephedrine, monoacetylmorphine, morphine, naloxone, nitrazepam, oxycodone, phenobarbital, pholcodine, pindolol, propranolol, viloxazine
Noninterfering: alprazolam, amitriptyline, brotizolam, butryptiline, camazepam, chlordiazepoxide, clobazam, clomipramine, clonazepam, clotiazepam, cloxazolam, desipramine, diazepam, dothiepin, doxepin, ethyl loflazepate, flunitrazepam, fluoxetine, flurazepam, fluvoxamine, halazepam, imipramine, lofepramine, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, melitracen, mianserin, midazolam, nordazepam, nortriptyline, opipramol, oxazepam, paroxetine, sertraline, temazepam, trazodone, triazolam, trimipramine
Interfering: thebacon

KEY WORDS
SPE

REFERENCE
Clauwaert,K.M.; Van Bocxlaer,J.F.; Lambert,W.E.; De Leenheer,A.P. Analysis of cocaine, benzoylecgonine, and cocaethylene in urine by HPLC with diode array detection, *Anal.Chem.*, **1996**, *68*, 3021–3028.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 3 mL 5 M NaOH, vortex 30 s, add 12 mL diethyl ether, rotate for 5 min, centrifuge at 2500 rpm for 5 min. Remove the ether layer and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C18
Mobile phase: MeOH:water 50:50 containing 7 mL/L butylamine, adjusted to pH 3.2 with sulfuric acid
Flow rate: 1.8
Injection volume: 50
Detector: E, Bioanalytical Systems Model LC4B, dual glassy carbon working electrode cell half operated in the parallel mode + 1.0 V and +0.9 V, stainless steel auxiliary electrode cell half, Ag/AgCl reference electrode. The detector was preceded by a Photronix Model 816 UV irradiator which irradiated the mobile phase in a 9.144 m length of 0.5 mm i.d. × 1.6 mm o.d. Teflon tubing in a three-dimensional figure eight configuration. The irradiation apparatus was maintained at 0-5° using an ice bath.

CHROMATOGRAM
Retention time: 6
Limit of detection: 50 ppb

OTHER SUBSTANCES
Simultaneous: methylphenidate, phenobarbital, nitrazepam
Interfering: chlordiazepoxide

REFERENCE

Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, *23*, 499–508.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B

Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 50:50 over 15 min.

Flow rate: 1.5

Injection volume: 20

Detector: UV 190-370

CHROMATOGRAM

Retention time: 10.5

OTHER SUBSTANCES

Extracted: morphine, ephedrine, phenylpropanolamine, diphenhydramine, nortriptyline, lidocaine, benzoylecgonine, norpropoxyphene, nordiazepam

Also analyzed: amitriptyline, amphetamine, meperidine, codeine, (different gradient)

REFERENCE

Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

SAMPLE

Matrix: vitreous humor

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH, 1 mL water, and 500 μL 10 mM pH 3.4 phosphoric acid. 500 μL Vitreous humor + 250 μL 10 mM phosphoric acid, add to SPE cartridge, wash with 1 mL 10 mM phosphoric acid, wash with 500 μL 100 mM acetic acid, wash with 500 μL MeOH, wash with 500 μL 300 mM ammonium hydroxide, elute with 2 mL MeOH. Evaporate eluate to dryness at 70° under nitrogen, reconstitute in 500 μL 20 μg/mL tetracaine hydrochloride in MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 Lichrospher 100 RP-18

Column: 125 × 4 Lichrospher 100 RP-18

Mobile phase: MeOH:buffer 75:25 (Buffer was 320 mL 20 mM K_2HPO_4 + 680 mL 20 mM KH_2PO_4, pH 7.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 235

CHROMATOGRAM

Retention time: 6.40

Internal standard: tetracaine (9.40)

Limit of detection: 500 ng/mL

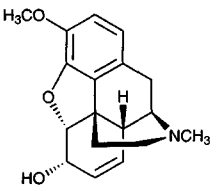
OTHER SUBSTANCES

Extracted: benzoylecgonine

KEY WORDS
SPE

REFERENCE
Fernández,P.; Rodríguez,P.; Bermejo,A.M.; López-Rivadulla,M.; Cruz,A. Simultaneous determination of cocaine and benzoylecgonine in vitreous humor by HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 883–890.

Codeine



Molecular formula: $C_{18}H_{21}NO_3$

Molecular weight: 299.37

CAS Registry No.: 76-57-3, 6069-47-8 (monohydrate), 5913-71-3 (acetate), 125-25-7 (HBr), 1422-07-7 (HCl), 6020-73-1 (salicylate), 125-27-9 (methyl bromide), 52-28-8 (phosphate), 41444-62-6 (phosphate hemihydrate), 1420-53-7 (sulfate), 6854-40-6 (sulfate trihydrate)

Merck Index: 2525

Lednicer No.: 1 287; 2 317

SAMPLE

Matrix: blood

Sample preparation: Condition a Certify SPE cartridge (Varian) with 2 mL MeOH and 2 mL 100 mM pH 8.0 phosphate buffer. Vortex 500 μL plasma and 1 mL 100 mM pH 8.0 phosphate buffer for 10 min and add the sample to the SPE cartridge using a gentle vacuum. Wash with 5 mL 100 mM pH 8.0 phosphate buffer, 1 mL water, 2 mL 100 mM pH 4.0 acetate buffer, and 2 mL MeOH. Dry cartridge under vacuum, wash with 200 μL butyl chloride:isopropanol 80:20, dry under vacuum. Elute with 1.2 mL butyl chloride: isopropanol 80:20, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 150 μL aliquot.

HPLC VARIABLES

Guard column: GuardPak C18 μBondapak

Column: 100 × 8 NovaPak C18

Mobile phase: MeCN:MeOH:13.3m M pH 7.5 phosphate buffer 2:23:75 containing 40 mg/L cetyltrimethylammonium bromide

Flow rate: 1

Injection volume: 150

Detector: E, Waters Model 460, working electrode 1.10-1.25 V, reference electrode KCl

CHROMATOGRAM

Retention time: 14.7

Internal standard: codeine

OTHER SUBSTANCES

Extracted: oxycodone

KEY WORDS

plasma; SPE; codeine is IS

REFERENCE

Wright,A.W.E.; Lawrence,J.A.; Iu,M.; Cramond,T.; Smith,M.T. Solid-phase extraction method with high-performance liquid chromatography and electrochemical detection for the quantitative analysis of oxycodone in human plasma, *J.Chromatogr.B*, **1998**, *712*, 169–175.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg ethyl SPE cartridge (J.T.Baker) with 2 mL MeOH, 1 mL water, and 2 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer. Mix 1 mL serum with 200 μL 1 μg/mL IS in water. Add to the SPE cartridge, wash with 1 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm Supelcosil LC-Si (Supelco)
Mobile phase: MeCN:MeOH:water:formic acid 5.2:59.8:34.65:0.35
Flow rate: 0.23
Injection volume: 5
Detector: MS, API I MS single quadrupole, ionspray, capillary tip 5000 V, interface plate 650 V, source 60°, positive ion mode, SIM, orifice 70 V, m/z 300

CHROMATOGRAM
Retention time: 22.09
Internal standard: nalorphine (15.40)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Extracted: - diamorphine, morphine

KEY WORDS
serum; pharmacokinetics; SPE; mouse

REFERENCE
Zuccaro,P.; Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; D'Ascenzo,G. Simultaneous determination of heroin, 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography-atmospheric pressure ionspray-mass spectrometry, *J.Anal.Toxicol.*, **1997**, *21*, 268–277.

SAMPLE
Matrix: blood, CSF, urine, vitreous humor
Sample preparation: Condition a 200 mg Bond Elut SPE cartridge with 1 mL MeOH, 1 mL water, and 2 mL buffer. Centrifuge 1.5 mL serum, CSF, urine, or vitreous humor at 14000 g for 5 min, vortex 1 mL supernatant with 2 mL buffer and 100 ng internal standard. Centrifuge at 5000 g for 10 min, slowly add 2 mL supernatant to the SPE cartidge, wash with 2 mL buffer, dry under vacuum for 5 min. Elute with 500 μL MeOH:500 mM acetic acid 90:10 under gravity. Dry the eluate under a stream of nitrogen, reconstitute in 100 μL mobile phase, centrifuge at 14000 g for 4 min, inject a 10-20 μL aliquot. (Prepare buffer by adjusting pH of 900 mL 960 mg/L ammonium carbonate to 9.3 with concentrated ammonium hydroxide and 1 M ammonium hydroxide, make up to 1 L.)

HPLC VARIABLES
Column: 125 × 3 4 μm Superspher RP 18
Mobile phase: MeCN:50 mM pH 3 ammonium formate buffer 5:95
Flow rate: 0.6 for 4 min, to 1.1 over 3 min, maintain at 1.1 for 10 min
Injection volume: 10-20
Detector: MS, Finnigan MAT SSQ 7000 single quadrupole, 100-500u, 10 V, positive ion, sheath gas nitrogen pressure 70 p.s.i., auxiliary gas nitrogen 20 mL/min; heated vaporizer temperature 400°, heated capillary temperature 170°, corona current, 5 μ A, m/z 300

CHROMATOGRAM
Retention time: 9.5
Internal standard: codeine-d6
Limit of detection: 2.5 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 6-monoacetylmorphine, morphine

KEY WORDS
serum; SPE

REFERENCE

Bogusz,M.J.; Maier,R.-D.; Erkens,M.; Driessen,S. Determination of morphine and its 3- and 6-glucuronides, codeine, codeine glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1997**, *703*, 115–127.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 212.2

CHROMATOGRAM

Retention time: 4.975

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 μBondapak C18

Mobile phase: MeCN:water adjusted to pH 3 with phosphoric acid 70:30

Flow rate: 1

Injection volume: 10

Detector: UV 210

CHROMATOGRAM

Internal standard: codeine phosphate

OTHER SUBSTANCES

Simultaneous: fentanyl

KEY WORDS
codeine is IS

REFERENCE
Vanbever,R.; Le Boulengé,E.; Préat,V. Transdermal delivery of fentanyl by electroporation. I. Influence of electrical factors, *Pharm.Res.*, **1996**, *13*, 559–565.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (Alltech)
Mobile phase: MeCN:water 25:75 containing 15 μL triethylamine per 100 mL
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 9.7

OTHER SUBSTANCES
Also analyzed: diamorphine, fentanyl, meperidine, morphine

REFERENCE
Lichtman,A.H.; Meng,Y.; Martin,B.R. Inhalation exposure to volatilized opioids produces antinociception in mice, *J.Pharmacol.Exp.Ther.*, **1996**, *279*, 69–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.15

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

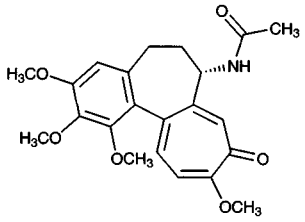
SAMPLE
Matrix: urine
Sample preparation: Dilute 200 μL rat urine with 1.8 mL water. 2 mL Human urine or diluted rat urine + 500 μL 25% ammonia, extract twice with 3 mL portions of cyclohexane: ethyl acetate 50:50 for 10 min. Centrifuge at 4000 rpm for 20 min, remove the organic layer and evaporate it under a stream of nitrogen. Reconstitute the residue in MeOH:50 mM pH 4.5 phosphate buffer 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 RP Select B RP8 (Merck)
Column: 250 × 4 5 μm RP Select B RP8 (Merck)
Mobile phase: Gradient. A was MeOH. B was THF. C was 50 mM pH 4.5 phosphate buffer. A:B:C from 27:3:70 to 22:3:75 over 18.9 min, to 48:4:48 over 10 min.
Flow rate: 0.5 for 18.9 min then 0.45
Injection volume: 20
Detector: UV 259

KEY WORDS
human; rat; codeine is IS

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

Colchicine



Molecular formula: $C_{22}H_{25}NO_6$
Molecular weight: 399.44
CAS Registry No.: 64-86-8
Merck Index: 2536
Lednicer No.: 1 152

SAMPLE

Matrix: bile, blood, gastric contents, tissue, vitreous humor
Sample preparation: Homogenize 10 g freshly minced liver with 10 mL water, adjust pH to 10 with 1 M NaOH, add 10 mg subtilisin (Sigma), heat at 55° for 1 h, adjust pH to 7.0 ± 0.5 with dilute mineral acid. 1 mL Liver homogenate, whole blood, plasma, bile, vitreous humor, or stomach contents + 1 mL 1 M $NaHCO_3$ + 7 mL dichloromethane, agitate gently for 30 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 µL 0.2% phosphoric acid, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: Novapak C18
Column: 150 × 4.6 5 µm Novapak C18
Mobile phase: MeCN:MeOH:buffer 13.4:26.6:60 (Buffer was 100 mM pH 6.0 KH_2PO_4 containing 5 µM pentanesulfonic acid.)
Flow rate: 0.8
Injection volume: 50
Detector: UV 245

CHROMATOGRAM

Retention time: 6.6
Limit of detection: 5 ng/mL

KEY WORDS

plasma; whole blood; liver

REFERENCE

McIntyre,I.M.; Ruszkiewicz,A.R.; Crump,K.; Drummer,O.H. Death following colchicine poisoning, *J.Forensic Sci.*, **1994**, *39*, 280–286.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 244

CHROMATOGRAM

Retention time: 3.07
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 4.0 mL Blood, plasma, or urine + 20 μL 1.0 μg/mL tofisopam in MeOH + 1.5 mL pH 8.0 dibasic ammonium phosphate + 4.5 mL dichloromethane, gently shake horizontally for 10 min, centrifuge at 3500 g for 10 min. Transfer the lower organic layer to 5 mL tube and evaporate under reduced pressure at 45° to 1.0 mL. Transfer into 1.5 mL Eppendorff-type plastic microtube and evaporate to dryness. Add 30 μL mobile phase, vortex for 10 s, centrifuge at 10 000 g for 5 min, inject a 0.6 μL aliquot of the

supernatant. (Equilibrate the column at least 3 h before analyzing. At the end of each chromatographic session clean column with MeCN:water 50:50 at 0.05 mL/min for 3 h.)

HPLC VARIABLES

Guard column: 1.0 × 0.8 5 μm C18 MGU-80 (LC Packing, Switzerland)
Column: 250 × 1.0 5 μm C18 Microbore (Alltech, USA)
Mobile phase: MeCN:2 mM pH 3.0 ammonium formate 75:25
Flow rate: 0.05
Injection volume: 0.6
Detector: MS, Perkin-Elmer Sciex API-100 double-quadrupole, OR +50 V, Q0 -10 V, IQ1 (lens) -12 V, ST (lens) -15 V, Q1 -13 V, EM +2200 V, TIC m/z 100-500 or 380-405, SIM m/z 400 ± 0.5 for colchicine, SIM m/z 383 ± 0.5 for tofisopam

CHROMATOGRAM

Retention time: 2.7
Internal standard: tofisopam (4.53)
Limit of detection: 0.6 ng/mL (SIM mode)

KEY WORDS

plasma; microbore; use PEEK tubing and injection loop

REFERENCE

Tracqui,A.; Kintz,P.; Ludes,B.; Rouge,C.; Douibi,H.; Mangin,P. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids, *J.Chromatogr.B*, **1996**, *675*, 235–242.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL 100 mg Bond Elut C2 SPE cartridge with one volume MeOH and two volumes of water. Dilute urine with four volumes of water. 500 μL Serum or diluted urine + 50 μL 2 μg/mL demecolcine in water, add to SPE cartridge, wash with 1 mL water, elute with three 350 μL portions of MeOH. Evaporate eluate to dryness under a stream of air at 40-50°, reconstitute in 150 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm C18
Column: 150 × 4.6 5 μm Microsorb C18
Mobile phase: MeCN:MeOH:buffer 13.4:26.6:60, adjusted to pH 6.0 with 100 mM KOH. After 10 min wash with MeCN:water 80:20 for 2 min, re-equilibrate for 12 min. (Buffer was 100 mM KH_2PO_4 containing 5 mM 1-pentanesulfonic acid.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 254 (plasma) or UV 350 (urine)

CHROMATOGRAM

Retention time: 9.41
Internal standard: demecolcine (8.20)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, N-desacetylcolchicine

KEY WORDS

serum; SPE

REFERENCE

Ko,R.J.; Li,W.Y.; Koda,R.T. Determination of the antimitotic agents N-desacetylcolchicine, demecolcine and colchicine in serum and urine, *J.Chromatogr.*, **1990**, *525*, 411–418.

SAMPLE

Matrix: blood, urine

Sample preparation: Add tofisopam to whole blood, plasma, or urine, adjust pH to 8.0, extract with dichloromethane.

HPLC VARIABLES

Column: 250 × 1 5 μm C18 Microbore (Alltech)

Mobile phase: MeCN:2 mM pH 3 ammonium formate buffer 75:25

Flow rate: 0.05

Detector: MS, Perkin-Elmer Sciex API-100, ionspray +4 kV, nebulizing gas nitrogen, curtain gas nitrogen, orifice +50 V, electron multiplier +2.2 kV, SIM m/z 400

CHROMATOGRAM

Retention time: 2.70

Internal standard: tofisopam (m/z 383) (4.53)

Limit of detection: 0.6 ng/mL

KEY WORDS

whole blood; plasma; microbore

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Determination of colchicine in human biofluids by HPLC-ISP-MS, *J.Anal.Toxicol.*, **1996**, *20*, 70–70.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 244

CHROMATOGRAM

Retention time: 13.118

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.3 7 μm Separon SGX
Mobile phase: 80 mM ammonium perchlorate in MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.18

OTHER SUBSTANCES
Simultaneous: amrinone, strychnine, nicotine, neostigmine, nicotine
Interfering: amitriptyline, promethazine, caffeine, procaine

REFERENCE
Eigendorf,H.G.; Nagel,S. Zur Analytik von Amrinone (Cordemcura). 2. Mitteillung: Hochdruckglüssigchromatographie [The analysis of amrinone (Cordemcura). 2. High pressure liquid chromatography], *Pharmazie*, **1987**, *42*, 631–631.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, gly-

benclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.09 (A), 4.15 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, di-

phenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

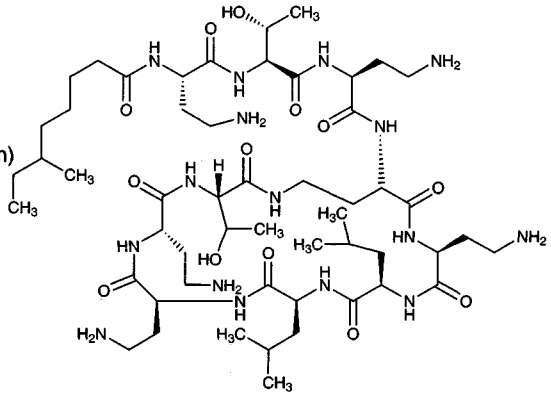
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Colistin



Molecular formula: $C_{58}H_{105}N_{16}Na_5O_{28}S_5$ (colistimethate sodium)

Molecular weight: 1749.85 (colistimethate sodium)

CAS Registry No.: 1066-17-7, 1264-72-8 (colistin sulfate), 8068-28-8 (colistimethate sodium), 21362-08-3 (colistimethate sodium)

Merck Index: 2542

SAMPLE

Matrix: bulk

Sample preparation: Make up solutions in 0.1% trifluoroacetic acid, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Ultrasphere C18

Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 100:0 over 25 min

Flow rate: 2

Detector: UV 215

CHROMATOGRAM

Retention time: 24 (two peaks)

OTHER SUBSTANCES

Simultaneous: colistin nonapeptide

REFERENCE

Warren,H.S.; Kania,S.A.; Siber,G.R. Binding and neutralization of bacterial lipopolysaccharide by colistin nonapeptide, *Antimicrob.Agents Chemother.*, **1985**, *28*, 107–112.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 400-500 μg/mL solution in water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil 5 C18

Mobile phase: MeCN:buffer 22:78 (Buffer was 23 mM phosphoric acid containing 10 mM acetic acid, and 50 mM sodium sulfate, adjust pH to 2.5 with triethylamine.)

Flow rate: 0.9

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 8 (colistin E2), 16 (colistin E1)

OTHER SUBSTANCES

Also analyzed: polymyxin B

REFERENCE

Elverdam,I.; Larsen,P.; Lund,E. Isolation and characterization of three new polymyxins in polymyxins B and E by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *218*, 653–661.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10-100 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ion-pair
Mobile phase: MeCN:water:Na_3PO_4 230:700:38 (v/v/w), adjust pH to 3.0 with phosphoric acid, make up to 1000 with water
Column temperature: 27
Flow rate: 1
Injection volume: 10
Detector: UV 185 or 200

CHROMATOGRAM
Retention time: 6 (E2), 9 (E1)
Limit of detection: 30 ng (UV 185)

OTHER SUBSTANCES
Simultaneous: polymyxin

REFERENCE
Whall,T.J. High-performance liquid chromatography of polymyxin B sulfate and colistin sulfate, *J.Chromatogr.*, **1981**, *208*, 118–123.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.8-8 μm), centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS 2
Mobile phase: MeCN:water:Na_2HPO_4 230:700:15.6 (v/v/w) adjusted to pH 3.2 with phosphoric acid, make up to 1000 with water
Column temperature: 28
Flow rate: 1.4
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 8.7 (E1 form)
Limit of detection: 3000 ng/mL

OTHER SUBSTANCES
Noninterfering: sucralfate

KEY WORDS
water

REFERENCE
Feron,B.; Adair,C.G.; Gorman,S.P.; McClurg,B. Interaction of sucralfate with antibiotics used for selective decontamination of the gastrointestinal tract, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2550–2553.

Copovithane

CAS Registry No.: 68045-74-9

SAMPLE

Matrix: blood

Sample preparation: Cool 2 mL plasma on ice for 5 min, add 200 μL 10 M perchloric acid, vortex vigorously, let stand on ice for 5 min, centrifuge at 17000 g for 15 min. Remove the supernatant and add it to 200 μL 10 M KOH, cool on ice for 5 min, centrifuge for 3 min. Remove the supernatant, add 2 mL hot (85°) saturated NaCl solution, add 3 mL chloroform, vortex vigorously, centrifuge at 17000 g for 15 min, repeat extract twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 5 M HCl, heat at 160° for 16 h, cool on ice, add 1 mL 5 M NaOH, add 3 mL 1 M pH 8.0 sodium bicarbonate buffer, add 750 μL 0.5% trinitrobenzenesulfonic acid in acetone, let stand in the dark for 2.5 h, extract three times with 3 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 250 μL 200 mM pH 6.4 Na_2HPO_4 in MeCN, inject an aliquot. (Copovithane is hydrolyzed to methylamine and the methylamine is derivatized.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm C18 (Waters)
Mobile phase: MeCN:water 30:70
Flow rate: 2
Detector: UV 340

CHROMATOGRAM

Retention time: 15
Limit of detection: 15 μg/mL

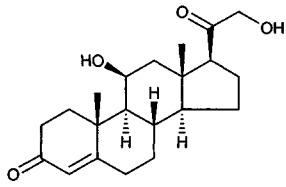
KEY WORDS

derivatization; plasma; pharmacokinetics

REFERENCE

Rosenblum,M.G.; Hortobagyi,G.N.; Wingender,W.; Hersh,E.M. Analysis of the antitumor agent Bay i 7433 (copovithane) in plasma and urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 159–166.

Corticosterone



Molecular formula: $C_{21}H_{30}O_4$
Molecular weight: 346.47
CAS Registry No.: 50-22-6
Merck Index: 2601

SAMPLE

Matrix: urine

Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 min, filter through a 0.22 μm filter unit. Dilute 0.5-1.5 mL urine to 4 mL with water. Add 40 ng IS. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH:10 mM NaOH 35:65, twice with 3 mL water and 3 mL MeOH:10 mM HCl 35:65. Elute with 3 mL absolute EtOH. Evaporate effluent under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 26.5:73.5
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 13.01
Internal standard: 11-desoxy-17hydroxycorticosterone (15.73)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; rat

REFERENCE

Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

Corticotropin

CAS Registry No.: 9002-60-2

Merck Index: 136

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl

Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min, maintain at 70:30 for 1 h. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)

Column temperature: 60

Flow rate: 0.3

Injection volume: 5-75

Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM

Retention time: 45

Limit of detection: 100 fmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin, vasopressin

KEY WORDS

plasma; SPE; post-column reaction

REFERENCE

Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was 0.08% trifluoroacetic acid. B was MeCN:0.08% trifluoroacetic acid 70:30. A:B from 70:30 to 50:50 over 30 min.

Flow rate: 1

Detector: UV 206

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: adrenocorticotropic hormone fragments, melanotropin

KEY WORDS
human

REFERENCE
McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Aquapore RP 300 (Kontron)
Mobile phase: Gradient. A was 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid. B was MeOH. A:B from 90:10 to 35:65 over 180 min.
Flow rate: 1
Injection volume: 100
Detector: UV 225

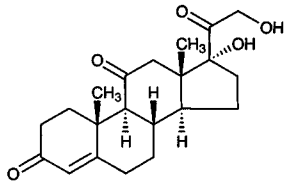
CHROMATOGRAM
Retention time: 145

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin hormone fragments, lipotropic hormone and fragments, melanotropin, endorphins, prolactin, somatropin, menotropins

KEY WORDS
pig

REFERENCE
Richter,W.O.; Schwandt,P. Separation of neuropeptides by HPLC: evaluation of different supports, with analytical and preparative applications to human and porcine neurophysins, β-lipotropin, adrenocorticotropic hormone, and β-endorphin, *J.Neurochem.*, **1985**, *44*, 1697–1703.

Cortisone



Molecular formula: $C_{21}H_{28}O_5$
Molecular weight: 360.45
CAS Registry No.: 53-06-5, 50-04-4 (acetate), 509-00-2 (21β-cyclopentanepropionate), 508-95-2 (phosphate)
Merck Index: 2602
Lednicer No.: 1 188, 190

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Centrifuge serum or amniotic fluid for 10 min. 0.5-1 mL Serum or amniotic fluid + 500 μL MeOH:water 5:95, mix, inject 750 μL onto column A with mobile phase A, after 5 min elute contents of column A onto column B with mobile phase B, monitor effluent from column B.

HPLC VARIABLES

Column: A Serumont-25 (Sekisui); B 260 × 4.6 5 μm Medipola-ODS C18 (Sekisui)
Mobile phase: A water; B MeCN:MeOH:buffer 2:7:20 (Buffer was 6.8 g/L KH_2PO_4, pH adjusted to 3.1 with concentrated phosphoric acid.)
Column temperature: 40
Flow rate: A 0.8; B 1
Injection volume: 750
Detector: UV 245

CHROMATOGRAM

Retention time: 56.8
Limit of detection: 5.8 ng

OTHER SUBSTANCES

Extracted: estetrol, estriol, hydrocortisone
Noninterfering: corticosterone, testosterone, hydroxyprogesterone, androstenedione, progesterone

KEY WORDS

serum; column-switching

REFERENCE

Noma,J.; Hayashi,N.; Sekiba,K. Automated direct high-performance liquid chromatographic assay for estetrol, estriol, cortisone and cortisol in serum and amniotic fluid, *J.Chromatogr.*, **1991**, *568*, 35–44.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge. Mix 1 mL plasma with 134.0 ng hydrocortisone-d_5 and 74.56 ng cortisone-d_5. Add the sample to the SPE cartridge, wash with 8 mL water, elute with 4 mL ethyl acetate, evaporate the eluate to dryness at 70° under a stream of nitrogen, dissolve the residue in 30 μL mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.0 4 μm LiChroCART Superspher 100
Mobile phase: A MeOH:THF:50 mM ammonium formate 17:53:180; B MeCN:50 mM ammonium formate 35:65
Flow rate: 0.6 (A); 1.3 (B)

Injection volume: 20
Detector: MS, Shimadzu LCMS-QP1000EX Model 750 B, thermospray, vaporizer control 155°, vaporizer tip 195°, vapor 274°, ion source block 295°, tip heater 305°, m/z 361

CHROMATOGRAM
Retention time: 10 (A)
Internal standard: hydrocortisone-d_5, cortisone-d_5
Limit of detection: 0.50 ng

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisolone, prednisone

KEY WORDS
plasma; SPE

REFERENCE
Shibasaki,H.; Furuta,T.; Kasuya,Y. Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution, *J.Chromatogr.B*, **1997**, *692*, 7–14.

SAMPLE
Matrix: blood
Sample preparation: Prepare a Bond-Elut C18 SPE column by washing with 2 mL MeCN, 2 mL acetone:water 2:98, and 4 mL water. Do not allow column to run dry. 2 mL Plasma + 40 μL 5 μg/mL dexamethasone in MeOH, add to SPE cartridge, allow to sit for 15 min, wash twice with 2 mL water, wash twice with 2 mL acetone:water 2:98, pull a vacuum on the column for 15 min, elute with 1 mL MeCN under vacuum. Evaporate the eluate to dryness under a stream of nitrogen at 40°, dissolve the residue in 150 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb Si-60
Mobile phase: Dichloromethane:water-saturated dichloromethane:THF:MeOH:glacial acetic acid 664.5:300:10:25:0.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Internal standard: dexamethasone (23.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: prednisolone acetate, prednisone, hydrocortisone, prednisolone

KEY WORDS
plasma; normal phase; pig; SPE

REFERENCE
Prasad,V.K.; Ho,B.; Haneke,C. Simultaneous determination of prednisolone acetate, prednisolone, prednisone, cortisone and hydrocortisone in swine plasma using solid-phase and liquid-liquid extraction techniques, *J.Chromatogr.*, **1986**, *378*, 305–316.

SAMPLE
Matrix: blood

Sample preparation: Condition a Tef Elutor C18 cartridge with two 3 mL portions of MeOH then two 3 mL portions of water. 1 mL Plasma + 50 μL 400 ng/mL flumethasone in 5:95 MeOH:water, heat at 50° for 10 min, add to cartridge, wash with 2 mL water, 1 mL MeOH:water 10:90, 4 mL acetone:water 20:80, apply suction to cartridge for 10 min to air dry. Elute with 1 mL MeOH, evaporate eluent at 45° under nitrogen, reconstitute with 50 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm C18 Hypersil
Mobile phase: MeCN:THF:water 8:10:82, containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 2.90
Internal standard: flumethasone (11.50)
Limit of detection: 300 pg/mL

OTHER SUBSTANCES

Simultaneous: prednisone, hydrocortisone, adrenosterone, prednisolone, estriol, corticosterone, methylprednisolone, dexamethasone, hydroxyprogesterone, testosterone, deoxycorticosterone, fluorometholone, spironolactone, equilenin, estrone, estradiol, progesterone, diphenhydramine, propranolol, aspirin, theophylline, imipramine, desipramine, indomethacin, amitriptyline, nortriptyline, nordiazepam, diazepam, chlordiazepoxide
Noninterfering: caffeine, nicotine, cotinine, chlorothiazide, acetazolamide, phenytoin, pheniramine, cephalothin, primidone, acebutolol, hydrochlorothiazide, quinine, acetophenetidine, furosemide, aldosterone, triamcinolone, ephedrine, allopurinol, phenylephrine
Interfering: tripelennamine, carbamazepine, probenecid, phenobarbital

KEY WORDS

plasma; SPE

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography, *Clin.Chem.*, **1992**, *38*, 346–352.

SAMPLE

Matrix: blood
Sample preparation: Condition a 2 mL 200 mg Tef Elutor C18 SPE cartridge (Versa Prep) with 3 mL MeOH and two 3 mL portions of water. 1 mL Plasma + 50 μL 400 ng/mL flumethasone in MeOH:water 5:95, heat at 50° for 10 min, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeOH:water 10:90, wash with 4 mL acetone:water 20:80, air-dry for 10 min, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm Hypersil
Mobile phase: MeCN:THF:water 8:10:82 containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 4

Internal standard: flumethasone (13)
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES

Extracted: hydrocortisone, corticosterone
Simultaneous: acebutolol, acetazolamide, acetophenetidin, adrenosterone, aldosterone, amitriptyline, androsten-3,17-dione, aspirin, cephalothin, chlordiazepoxide, chlorothiazide, dehydrocorticosterone, deoxycorticosterone, deoxycortisol, desipramine, dexamethasone, diazepam, diphenhydramine, equilenin, estradiol, estriol, estrone, fluorometholone, furosemide, hydrochlorothiazide, hydroxycorticosterone, hydroxyprogesterone, hydroxyprogesterone, imipramine, indomethacin, methylhydroxyprogesterone, methylprednisolone, nandrolone, nordiazepam, nortriptyline, pheniramine, phenobarbital, phenytoin, prednisolone, prednisone, primidone, probenecid, progesterone, propranolol, quinine, spironolactone, testosterone, theophylline, triamcinolone
Noninterfering: caffeine, nicotine, cotinine, ephedrine, allopurinol, phenylephrine
Interfering: tripelennamine, carbamazepine

KEY WORDS

serum; SPE

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Assay of human plasma cortisone by liquid chromatography: normal plasma concentrations (between 8 and 10 a.m.) of cortisone and corticosterone, *J.Chromatogr.*, **1993**, *613*, 195–201.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL IS in water, extract twice by shaking for 1 min with 1.2 mL dichloromethane, evaporate organic layer below 40° under reduced pressure, dissolve residue in 100 μL MeCN. Add 10 μL reagent 1, add 10 μL reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μL water, add 200 μL MeOH:water 1:1, add to Sep-Pak C18 cartridge, wash vial with 2 mL MeOH:water 1:1 and add washings to cartridge, wash cartridge with 40 mL MeOH:water 1:1, elute with 5 mL MeOH. Concentrate eluent to 500 μL by evaporation at 40° under reduced pressure, inject 20 μL aliquot. (Reagent 1 was 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, add 700 mg 4-piperidinopyridine, dilute to 10 mL with MeCN. Reagent 2 was 700 mg 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in 10 mL MeCN. Prepare 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as follows. Add 13 g 4-carboxybenzaldehyde (terephthalaldehydic acid) in 400 mL EtOH dropwise to 4,5-dimethyl-1,2-phenylenediamine in 400 mL EtOH in an ice bath, after 1 h reflux for 8 h, cool to room temperature, collect the precipitate, recrystallize three times from MeOH:water 50:50 to give 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as a white amorphous product (mp >300°) (J.Chromatogr. 1991, 585, 219). 4-Piperidinopyridine is not commercially available but 4-dimethylaminopyridine or 4-pyrrolidinopyridine can be used instead although interferences are greater (J. Chromatogr. 1991, 585, 219). Alternatively 4-piperidinopyridine can be synthesized as follows. Add 200 mmoles piperidine dropwise with stirring to 15 g phosphorus pentoxide and 9.51 g 4-hydroxypyridine, heat at 250° for 7 h, cautiously pour onto 200 g ice, add 400 mL 1 M NaOH, add 200 mL ether. Remove the ether layer and extract the aqueous layer three times with 100 mL portions of ether. Combine the organic layers and dry them over anhydrous potassium carbonate, evaporate, distil the residue, recrystallize from petroleum ether (bp 80-100°) to give 4-piperidinopyridine (bp 167-170°/11 mm Hg; mp 79-80°) (Synthesis 1978, 844). Alternatively, add 1.94 g 4-bromopyridine hydrochloride to 5 mL 50% NaOH, add 5 mL piperidine, add 2.72 g benzyltriethylammonium bromide, heat at 100° for 5 h, remove excess piperidine by distillation, add 25 mL water, extract four times with 25 mL portions of benzene. Combine the organic layers and dry them over anhydrous sodium sulfate, boil the residue with petroleum ether to give 4-piperidinopyridine (mp 80°) (Syn. Commun. 1979, 9, 251). Prepare 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate as follows. Stir 1.41 moles isopropy-

lisocyanate in 750 mL dichloromethane at 5°, add 144 g 3-dimethylaminopropylamine (N,N-dimethyl-1,3-propanediamine) in 250 mL dichloromethane at such a rate that the temperature does not exceed 10°, add 500 mL triethylamine, add 300 g p-toluenesulfonyl chloride in 300 mL dichloromethane at such a rate that the temperature does not exceed 10°, reflux for 3 h, add 400 g anhydrous sodium carbonate, add 3.5 L ice water, stir vigorously for 30 min, remove the organic phase. Extract the aqueous phase three times with 500 mL portions of dichloromethane. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate under reduced pressure, distil the residue to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide (bp 91-92°/10 mm Hg (Ber. 1941, 74B, 1285)) (cf. Org. Syn. 1973, Coll. Vol. V, 555). Prepare pyridine perchlorate from pyridine and 20% perchloric acid, crystallize from EtOH (Ber. 1926, 59, 446). Add 18 g pyridine perchlorate in portions to 100 mmoles 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide stirred in 200 mL dichloromethane at 0°, let stand for 30 min, filter, add 200 mL anhydrous diethyl ether to the filtrate. Filter off the precipitate and recrystallize it from dichloromethane/diethyl ether to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate (mp 88-90°) (Chem. Pharm. Bull. 1985, 33, 5375).)

HPLC VARIABLES

Guard column: 50 × 4.6 7 μm Zorbax ODS
Column: 250 × 4.6 7 μm Zorbax ODS
Mobile phase: MeOH:water 75:25 containing 5 mM tetramethylammonium hydrogen sulfate
Flow rate: 0.4
Injection volume: 20
Detector: F ex 334 em 418

CHROMATOGRAM

Retention time: 25.2
Internal standard: fluocinolone acetonide (40.7)
Limit of detection: 0.6-3 pg/mL

OTHER SUBSTANCES

Simultaneous: aldosterone, corticosterone, hydrocortisone, dexamethasone, triamcinolone

KEY WORDS

plasma; derivatization

REFERENCE

Katayama,M.; Masuda,Y.; Taniguchi,H. Determination of corticosteroids in plasma by high-performance liquid chromatography after pre-column derivatization with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole, *J.Chromatogr.*, **1993**, *612*, 33–39.

SAMPLE

Matrix: blood
Sample preparation: Prepare a Sep-Pak Plus Environmental C18 cartridge by washing with 15 mL MeOH then 15 mL water. 1 mL Serum + 100 μL 3 μg/mL betamethasone in isopropanol:MeCN 1:1 + 100 μL isopropanol:acetonitrile 1:1, mix, add to SPE cartridge, wash with 10 mL water, elute with 3 mL MeOH. Evaporate the eluate at 50° under a stream of nitrogen, reconstitute in 200 μL mobile phase A, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18 guard column
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. A was isopropanol:50 mM pH 4.5 acetate buffer 10:90. B was isopropanol:50 mM pH 4.5 acetate buffer 30:70. A:B from 90:10 to 30:70 over 25 min, hold at 30:70 for 5 min, to 90:10 over 5 min, hold at 90:10 for 15 min before next injection.
Column temperature: 40
Flow rate: 1

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 24
Internal standard: betamethasone (33)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, prednisone, hydrocortisone, prednisolone

KEY WORDS
serum; SPE

REFERENCE
Hirata,H.; Kasama,T.; Sawai,Y.; Fike,R.R. Simultaneous determination of deflazacort metabolites II and III, cortisol, cortisone, prednisolone and prednisone in human serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 55–61.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2500 g for 10 min, mix the supernatant with an equal volume of 1 M pH 3.0 glycine buffer containing 0.2% Tween 20, centrifuge at 2500 g for 10 min, inject an aliquot of the supernatant on to column A and elute to waste with mobile phase, after 3 min divert the effluent from column A on to column B, after 3 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with mobile phase for 28 min.

HPLC VARIABLES
Column: A 30 × 2.1 Spherisorb C1 pH stable; B 150 × 2.1 Spherisorb C1 pH stable
Mobile phase: 5 mM pH 7.3 Tris-nitric acid buffer containing 0.1% Tween 20 and 150 mM sodium nitrate
Column temperature: 40
Flow rate: 0.2
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisolone

KEY WORDS
plasma; column-switching; heart-cut

REFERENCE
Lövgren,U.; Johansson,M.; Kronkvist,K.; Edholm,L.-E. Biocompatible sample pretreatment for immunochemical techniques using micellar liquid chromatography for separation of corticosteroids, *J.Chromatogr.B*, **1995**, *672*, 33–44.

SAMPLE
Matrix: blood
Sample preparation: Condition an Empore C8 extraction disc SPE cartridge (3M Co.) by adding 500 μL MeOH and forcing through three drops, discard the remaining liquid, add water, force through three drops, discard the water. 300 μL Serum + 150 μL IS solution, let stand at room temperature for 10 min, add 800 μL saturated sodium borate solution,

mix, centrifuge at 12400 g for 3 min (if necessary), add to SPE cartridge, centrifuge at 100-120 g for 5 min, force through 200 μL water, force through 500 μL MeOH:water 18:82, elute with 50 μL MeCN then 150 μL water, mix the eluates, inject a 20 μL aliquot. (IS solution contained 0.5 mg/L fludrocortisone and 0.75 mg/L methylprednisolone in 400 mM HCl.) (The extraction disc permits use of lower volumes of eluate than a conventional SPE cartridge.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (Du Pont)
Column: 250 × 2 Ultrasphere C18 or 250 × 4.6 Ultrasphere C18
Mobile phase: THF:water 20:80 (Use a 150 × 4.6 37-53 μm silica gel (Whatman) saturating column (held at 55°) between the pump and the injector.)
Column temperature: 55
Flow rate: 0.18 (250 × 2 column) or 0.8 (250 × 4.6 column)
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Internal standard: fludrocortisone (15), methylprednisolone (20)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisone, prednisolone, corticosterone
Simultaneous: aldosterone, triamcinolone, metyrapone, 11-deoxycortisol, dexamethasone, 21-deoxycortisone, androsteindione, beclomethasone, 11-deoxycorticosterone, testosterone, 17-hydroxyprogesterone, progesterone, pregnenolone

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KEY WORDS
serum; SPE; extraction disc

REFERENCE
Lensmeyer,G.L.; Onsager,C.; Carlson,I.H.; Wiebe,D.A. Use of particle-loaded membranes to extract steroids for high-performance liquid chromatographic analyses. Improved analyte stability and detection, *J.Chromatogr.A*, **1995**, *691*, 239–246.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 14.73

Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, dexamethasone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Urine. 1 mL Urine + 1 mL MeOH:EtOH 50:50, centrifuge at 4000 g for 10 min. Remove the supernatant and evaporate to about 200 μL under a stream of nitrogen at 37°, inject a 5-20 μL aliquot. Plasma. Mix plasma with an equal volume of MeOH:EtOH 50:50, let stand at -20° for 30 min or overnight. Remove supernatant and wash precipitate twice with equal volumes of MeOH:EtOH 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL MeOH:water 65:35, inject a 5-20 μL aliquot. Tissue. Homogenize-(Polytron) fetal tissue in 10-15 mL MeOH:dimethoxymethane 50:50 for 1 min or until breakup was complete, shake at 37° overnight, centrifuge at 4000 g for 5 min. Filter (Whatman No. 1 filter paper) supernatant. Resuspend precipitate in MeOH:dimethoxymethane 50:50, filter, wash precipitate with MeOH. Combine filtrates, evaporate to dryness under nitrogen, resuspend residue in up to 500 μL MeOH:water 65:35, centrifuge, inject a 5-20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 70 × 6 35-50 μm Bondapak C18 Corasil
Column: 250 × 10 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeOH:10 mM pH 6.9 ammonium acetate from 10:90 to 100:0 over 50 min (Waters No. 5 convex gradient)
Flow rate: 1.5
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM
Retention time: 29.39

OTHER SUBSTANCES
Extracted: metabolites, triamcinolone, triamcinolone acetonide, hydrocortisone, cortexolone, 6β-hydroxycortisol, cortisol glucuronide

KEY WORDS
plasma; monkey

REFERENCE
Althaus,Z.R.; Rowland,J.M.; Freeman,J.P.; Slikker,W.,Jr. Separation of some natural and synthetic corticosteroids in biological fluids and tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *227*, 11–23.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 7 mL MeOH and 14 mL water. Add 40 ng 6β-hydroxycortisone to 400 μL plasma or urine, add the mixture to the SPE cartridge, wash with 6 mL water, 3 mL MeOH:water 12:88, and 3 mL petroleum ether, elute with 5 mL ethyl acetate. Dry the eluate under reduced pressure at 40°, add 200 μL MeCN:triethylamine 90:10 and MeCN:0.1% quinuclidine 20:80 to the residue, vortex. Add 200 μL 0.02% 9-anthroyl nitrile and a few molecular sieves (4A), let stand for 30 min, evaporate under reduced pressure at 40°, dissolve the residue in 200 μL acetone, dilute with 2 mL n-hexane. Add the mixture to a Sep-Pak Plus Silica SPE cartridge, wash with 14 mL 1,2-dichloroethane, elute with 5 mL ethyl acetate. Evaporate the eluate under reduced pressure at 40°, reconstitute the residue in 200 μL mobile phase, inject a 30-60 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Japan)

Mobile phase: Dioxane:ethyl acetate:chloroform:n-hexane:pyridine 58.1:11.6:11.6:16.3:2.4 (Caution! Dioxane and chloroform are carcinogens!)

Flow rate: 1 for 45 min, to 1.2 over 5 min

Injection volume: 30-60

Detector: F ex 360 em 460

CHROMATOGRAM

Retention time: 28

Internal standard: 6β-hydroxycortisone (86)

Limit of detection: 100 pg/mL

OTHER SUBSTANCES

Extracted: prednisone, prednisolone, hydrocortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone

KEY WORDS

derivatization; plasma; urine; SPE; normal phase

REFERENCE

Shibata,N.; Hayakawa,T.; Takada,K.; Hoshino,N.; Minouchi,T.; Yamaji,A. Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile, *J.Chromatogr.B*, **1998**, *706*, 191–199.

SAMPLE

Matrix: formulations

Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH:water 75:25

Flow rate: 1.5

Injection volume: 10

Detector: UV 240

CHROMATOGRAM

Retention time: 3.3

Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine

Interfering: formebolone, benzyl alcohol, testolactone

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: perfusate

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. 3 mL Perfusate + 500 ng 6α-methylprednisolone, add to the SPE cartridge, wash three times with 10 mL aliquots of water, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-18
Column: two 250 × 4.6 Spheri-5 RP-18 columns in series
Mobile phase: MeOH:water 53:47
Column temperature: 40
Flow rate: 1.1
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 16
Internal standard: 6α-methylprednisolone (30)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: hydrocortisone, dihydrocortisone, dihydrocortisol, metabolites
Simultaneous: prednisolone
Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, carbamazepine, cholesterol, clonazepam, dehydroepiandrosterone, dexamethasone, diazepam, estradiol, estriol, hydroxyprogesterone, methimazole, phenobarbital, prednisone, progesterone, ritodrine, scopolamine, testosterone

KEY WORDS

SPE

REFERENCE

Dodds,H.M.; Maguire,D.J.; Mortimer,R.H.; Addison,R.S.; Cannell,G.R. High performance liquid chromatographic separation of cortisol, cortisone, and their 20-reduced metabolites in perfusion media, *J.Liq.Chromatogr.*, **1995**, *18*, 1809–1820.

SAMPLE
Matrix: solutions
Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 5 μm octadecyl Bakerbond
Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin
Column temperature: 5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 0.8

OTHER SUBSTANCES
Simultaneous: hydrocortisone, testosterone, prednisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE
Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm SI-100 (Brownlee)
Mobile phase: Butyl chloride:THF:MeOH:glacial acetic acid 95:7:3.5:3 (Butyl chloride was 50% water saturated.)
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (cortisone), 8.5 (cortisone acetate)

OTHER SUBSTANCES
Simultaneous: hydrocortisone acetate, hydrocortisone, 4-androstene-3,11,17-trione

KEY WORDS
normal phase

REFERENCE
Kane,M.P.; Tsuji,K. Radiolytic degradation scheme for ^{60}Co-irradiated corticosteroids, *J.Pharm.Sci.*, **1983**, *72*, 30–35.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38

Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.19 (cortisone acetate)

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, 17α-hydroxyprogesterone, dexamethasone acetate, estradiol, estrone, progesterone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 34–38.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution (eluate from preparative HPLC) to dryness under a stream of nitrogen, reconstitute with 10 μL 2 μg/mL 9-anthroylnitrile (Wako) in MeCN and 10 μL triethylamine:MeCN 30:70 under nitrogen, let stand at room temperature for 20 min, add 5 μL water, after 6 min add 50 μL 600 mM acetic acid in MeCN, evaporate to dryness under a stream of nitrogen at 37°, reconstitute with 90 μL MeOH: 0.4 N NaH_2PO_4 60:40, add to a Cyclobond I silica-bonded β-cyclodextrin SPE cartridge (Astec), wash with 1 mL water, wash with 8 mL MeOH:water 25:75 containing 7.5 mM pH 7.0 phosphate buffer, elute with 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot on to column A and elute to waste with mobile phase, after the solvent front has passed through divert the effluent from column A on to column B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 2.1 silica (Brownlee); B 150 × 2 Hypersil
Mobile phase: Hexane:ethyl acetate 67:33 (half-saturated with water)
Flow rate: 0.5
Detector: F ex 305-395 em 430-470

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisolone

KEY WORDS
derivatization; SPE; column-switching; normal phase

REFERENCE
Haegele,A.D.; Wade,S.E. Ultrasensitive differential measurement of cortisol and cortisone in biological samples using fluorescent ester derivatives in normal phase HPLC, *J.Liq.Chromatogr.*, **1991**, *14*, 1133–1148.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 17.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-1

Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: k' 0.9437

OTHER SUBSTANCES
Also analyzed: androsterone (UV 210), cortexolone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240), testosterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 μM solution in MeOH.

HPLC VARIABLES
Column: 470 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeOH:water 56:44
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: dehydrocorticosterone, hydrocortisone, methylprednisolone, prednisolone, prednisone, tetrahydrocortisol, tetrahydrocortisone

REFERENCE
Lukulay,P.H.; McGuffin,V.L. Comparison of solvent modulation with premixed mobile phases for the separation of corticosteroids by liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 4039–4062.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 100 ppm solution.

HPLC VARIABLES
Column: 150 × 4.6 Develosil ODS-5
Mobile phase: Gradient. MeOH:water from 50:50 to 90:10 over 15 min
Flow rate: 1
Injection volume: 10
Detector: MS, JEOL JMS-SX102A reversed geometry (BE), accelerating voltage +5 kV, air pressure chemical ionization APCI, nebulizer 290°, ion source chamber 400°, discharge electrode, skimmer 1 aperture 300 μm, skimmer 2 aperture 400 μm, no nebulizer gas

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: corticosterone, hydrocortisone, progesterone

REFERENCE
Nojima,K.; Fujimaki,S.; Hertsens,R.C.; Morita,T. Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer, *J.Chromatogr.A*, **1995**, *712*, 17–19.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was hexane. B was ethyl acetate. C was 0.1% formic acid in MeCN. D was 0.1% formic acid in water. A:B:C:D 100:0:0:0 for 5 min, to 0:100:0:0 over 15 min, maintain at 0:100:0:0 for 5 min, to 0:0:100:0 over 5 min, maintain at 0:0:100:0 for 5 min; to 0:0:0:100 over 25 min, maintain at 0:0:0:100 for 5 min.
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM
Retention time: 21.90

OTHER SUBSTANCES
Simultaneous: acetylcholine, cholesterol, choline, dextrose, estradiol, glycine, phenylalanine, sodium, testosterone

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 in, filter through a 0.22 μm filter unit. Dilute 0.75-3mL urine to 4 mL with water. Add 30 ng IS. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH: 10 mM NaOH 30:70, twice with 3 mL water and with 3 mL MeOH:10 mM HCl 30:70. Elute with 3 mL EtOH. Evaporate eluate under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 24:76
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 11.45
Internal standard: dexamethasone (22.01)
Limit of detection: 3.8 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, hydrocortisone

KEY WORDS
SPE; human; pig

REFERENCE
Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 1.5 μg betamethasone + 100 mg K_2HPO_4 + 500 mg anhydrous sodium sulfate + 5 mL diethyl ether, shake mechanically for 10 min, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:water from 4:96 to 30:70 over 10 min, to 45:55 over 5 min, to 50:50 over 3 min
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 246

CHROMATOGRAM
Retention time: 11.46
Internal standard: betamethasone (12.83)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, deoxycorticosterone, hydrocortisone, 11α-hydroxyprogesterone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide

REFERENCE
Park,S.-J.; Kim,Y.-J.; Pyo,H.-S.; Park,J. Analysis of corticosteroids in urine by HPLC and thermospray LC/MS, *J.Anal.Toxicol.*, **1990**, *14*, 102–108.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 μL 5 μg/mL IS in MeOH, inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeCN:water 32:68
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 5.3

Internal standard: methylprednisolone (9)

OTHER SUBSTANCES

Simultaneous: triamcinolone, triamcinolone acetonide, prednisone, dexamethasone, betamethasone, corticosterone, hydroxyprogesterone, fluorocortisone acetate, hydrocortisone, fluorocortisone
Interfering: prednisolone

KEY WORDS

SPE also discussed

REFERENCE

Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Solvent and solid-phase extraction of natural and synthetic corticoids in human urine, *J.Chromatogr.B*, **1994**, *652*, 83–89.

SAMPLE

Matrix: urine
Sample preparation: 3 mL Urine + 100 ng methylprednisolone + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 μL MeOH, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: MeCN:water 30:70
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 6.5
Internal standard: methylprednisolone (14)
Limit of detection: 55 pg

OTHER SUBSTANCES

Extracted: hydrocortisone
Simultaneous: fluorocortisone
Noninterfering: corticosterone, deflazacort, deoxycorticosterone, fluorocortisone acetate, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, prednisolone, prednisone, triamcinolone acetonide

REFERENCE

Santos-Montes,A.; Gonzalo-Lumbreras,R.; Izquierdo-Hornillos,R. Simultaneous determination of cortisol and cortisone in urine by reversed-phase high-performance liquid chromatography. Clinical and doping control applications, *J.Chromatogr.B*, **1995**, *673*, 27–33.

SAMPLE

Matrix: urine
Sample preparation: 10 mL Urine + 40 μL 25 μg/mL corticosterone, vortex briefly, add 1 mL 100 mM NaOH, vortex briefly, add 3 mL dichloromethane, rotate at 20 rpm for 45 min, centrifuge at 1000 g for 15 min, discard the aqueous layer, centrifuge at 1000 g for 10 min, discard the aqueous layer, add 150 mg NaCl, break up emulsion, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 150 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. MeOH:water from 30:70 to 44:56 over 6 min, maintain at 44:56 for 14 min, return to initial conditions over 3 min, re-equilibrate for 5 min.
Flow rate: 1
Detector: UV 246

CHROMATOGRAM

Retention time: 12.8
Internal standard: corticosterone (17.8)

OTHER SUBSTANCES

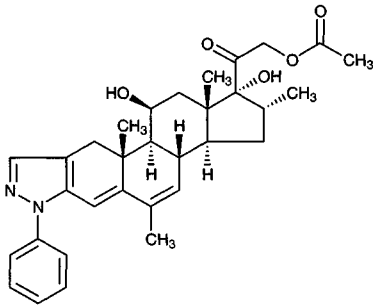
Extracted: hydrocortisone

REFERENCE

Lee,Y.S.; Lorenzo,B.J.; Koufis,T.; Reidenberg,M.M. Grapefruit juice and its flavonoids inhibit 11β-hydroxysteroid dehydrogenase, *Clin.Pharmacol.Ther.*, **1996**, *59*, 62–71.

Cortivazol

Molecular formula: $C_{32}H_{38}N_2O_5$
Molecular weight: 530.66
CAS Registry No.: 1110-740-3
Merck Index: 2603



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 12.002

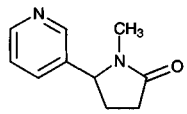
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cotinine



Molecular formula: $C_{10}H_{12}N_2O$

Molecular weight: 176.22

CAS Registry No.: 486-56-6, 5695-98-7 (fumarate)

Merck Index: 2619

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 4.7

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: meconium

Sample preparation: Condition a 3 mL C8 SPE cartridge (Backer-Bond, Gross Gerau, Germany) with 5 mL MeOH and 5 mL water. Condition a 3 mL silica SPE cartridge (Backer-Bond) with 5 mL chloroform. Place the C8 SPE cartridge on top of the silica SPE cartridge. Thaw and mix meconium, weigh a 2 g aliquot, add IS, and emulsify in 20 mL 100 mM pH 8.0 phosphate buffer. Vortex, centrifuge, filter the supernatant. Extract the supernatant 3 times with 2 mL portions of chloroform. Evaporate chloroform extracts to dryness and dissolve in 10 mL pH 9.0 boric buffer. Add to the SPE cartridges, remove the C8 column and elute the silica column with 3 mL MeOH:dichloromethane:ammonium hydroxide 30:70:1. Evaporate the eluate to dryness under nitrogen and redissolved in 100 μL water. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb C18
Mobile phase: MeCN:MeOH:water:pH 4.66 acetate buffer: acetic acid 2:29:50:20:1, pH adjusted to 4.3 with diethylamine
Flow rate: 1.0
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: 7.29
Internal standard: ephedrine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, nicotine

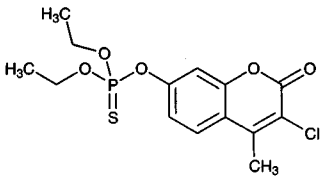
KEY WORDS

SPE

REFERENCE

Baranowski,J.; Pochopien,G.; Baranowska,I. Determination of nicotine, cotinine and caffeine in meconium using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *707*, 317–321.

Coumaphos



Molecular formula: $C_{14}H_{16}ClO_5PS$
Molecular weight: 362.77
CAS Registry No.: 56-72-4
Merck Index: 2626

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize mouse brain in ten volumes 100 mM pH 7.4 sodium phosphate buffer. 2 mL Plasma or homogenate + 1 g NaCl + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen.

HPLC VARIABLES

Column: 300 × 4 μPorasil
Mobile phase: Dichloromethane:glacial acetic acid 100:0.02
Flow rate: 1
Detector: UV 290

CHROMATOGRAM

Retention time: 10.0
Internal standard: coumaphos

OTHER SUBSTANCES

Extracted: parathion, chlorpyrifos

KEY WORDS

plasma; rat; mouse; microsomes; brain; coumaphos is IS

REFERENCE

Sultatos,L.G.; Costa,L.G.; Murphy,S.D. Determination of organophosphorus insecticides, their oxygen analogs and metabolites by high pressure liquid chromatography, *Chromatographia*, **1982**, *15*, 669–671.

SAMPLE

Matrix: eggs, milk
Sample preparation: Prepare a SPE column from 500 mg silanized Celite 545 followed by 5 g Nuchar S-N:silanized Celite 545 1:4 in a 22 mm i.d. chromatographic column, wash with 50 mL MeCN:toluene 75:25. Eggs. 150 g Chopped eggs + 300 mL MeCN, homogenize (Polytron) at half speed for 30 s and full speed for 1 min, vacuum filter (S & S 597 paper). Remove an aliquot equivalent to 100 g sample, make up to 100 mL with water, add 500 μL hexadecane to reduce foaming, concentrate to 75 mL on a rotary evaporator at 35°. Place mixture in a separatory funnel, add 15 g NaCl, shake until dissolved, wash the original flask with three 25 mL aliquots of MeCN, add the washings to the separatory funnel, shake 30 s, let separate for 5 min, remove the MeCN layer, extract the aqueous layer with 50 mL MeCN. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, add 50 mL 2% NaCl, extract with 100, 25, and 25 mL portions of dichloromethane. Combine lower organic layers and pass them through a 50 × 22 column of anhydrous sodium sulfate, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 10 mL dichloromethane, add to SPE column, wash in with 10 mL dichloromethane, wash in with 25 mL MeCN:toluene 75:25, elute with 100 mL MeCN:toluene 75:25 at 5 mL/min, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 5 mL MeOH, filter 5 μm, inject a 10 μL aliquot of the filtrate. Milk. 150 g Milk + 300 mL acetone, shake vigorously for 1 min, centrifuge at 1200 rpm for 5

min. Remove 277 mL supernatant and add it to 15 mL water, concentrate to 75 mL on a rotary evaporator at 35°. Place mixture in a separatory funnel, add 15 g NaCl, shake until dissolved, wash the original flask with three 25 mL aliquots of MeCN, add the washings to the separatory funnel, shake 30 s, let separate for 5 min, remove the MeCN layer, extract the aqueous layer with 50 mL MeCN. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, add 50 mL 2% NaCl, extract with 100, 25, and 25 mL portions of dichloromethane. Combine lower organic layers and pass them through a 50 × 22 column of anhydrous sodium sulfate, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 10 mL dichloromethane, add to SPE column, wash in with 10 mL dichloromethane, wash in with 25 mL MeCN:toluene 75:25, elute with 100 mL MeCN:toluene 75:25 at 5 mL/min, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 5 mL MeOH, filter 5 μm, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 70 × 2.1 25-37 μm Co:Pell ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water 12:88 to 70:30 over 30 min, then 100:0 for 5 min, re-equilibrate for 10 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: F ex 320 em 385

CHROMATOGRAM

Retention time: 31
Limit of detection: <10 ppb

KEY WORDS

SPE

REFERENCE

Krause,R.T.; Min,Z.; Shotkin,S.H. Determination of coumaphos and its oxygen analog in eggs and milk by using a multiresidue method with liquid chromatographic quantitation and capillary gas chromatographic/mass spectrometric confirmation, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 1353–1357.

SAMPLE

Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 313

CHROMATOGRAM

Retention time: 0.3
Limit of detection: 300 pg

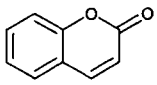
OTHER SUBSTANCES

Also analyzed: amitraz (UV 313), chlorpyrifos (UV 313), crotoxyphos (UV 229), permethrin (UV 229), phosmet (UV 229)

REFERENCE

Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

Coumarin



Molecular formula: $C_9H_6O_2$
Molecular weight: 146.15
CAS Registry No.: 91-64-5
Merck Index: 2630

SAMPLE

Matrix: blood, tissue
Sample preparation: Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 μg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH:chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 200 mm long μBondapak C18
Mobile phase: MeOH:0.8% acetic acid 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 3.9
Internal standard: N,N-diphenylbenzidine (9.3)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: bromadiolone, brodifacoum, coumatetralyl, warfarin

KEY WORDS

SPE; plasma; heart; lung; liver; kidney; spleen

REFERENCE

Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685–688.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

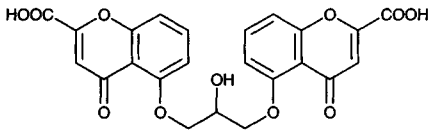
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cromolyn



Molecular formula: $C_{23}H_{16}O_{11}$, $C_{23}H_{14}Na_2O_{11}$ (disodium salt)
Molecular weight: 468.37
CAS Registry No.: 16110-51-3, 15826-37-6 (disodium salt)
Merck Index: 2658
Lednicer No.: 3 66, 235; 4 44, 137, 150, 205

SAMPLE

Matrix: blood, microsomal incubations, tissue
Sample preparation: Microsomal incubation. Add 200 μL microsomal incubation to 800 μL MeCN:DMSO:MeOH 60:5:35, vortex for 20 s, centrifuge. Dilute the supernatant two-fold with water, inject a 20 μL aliquot. Tissue. Homogenize tissue with 3 volumes MeCN:DMSO:MeOH 60:5:35, centrifuge, dilute with water, inject an aliquot.

HPLC VARIABLES

Guard column: Hypersil ODS-2 C8
Column: 100 × 4.0 Hypersil ODS-2 C8
Mobile phase: MeOH:4mM benzyltributylammonium chloride 48.5:51.5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 3.95
Limit of detection: ⩽ 30 ng/mol

KEY WORDS

lung; liver; kidney; human; dog; rat; rabbit; pharmacokinetics

REFERENCE

Saah,F.; Wu,W.-M.; Eberst,K.; Marvanyos,E.; Bodor,N. Design, synthesis, and pharmacokinetic evaluation of a chemical delivery system for drug targeting to lung tissue, *J.Pharm.Sci.*, **1996**, *85*, 496–504.

SAMPLE

Matrix: urine
Sample preparation: Dilute urine 10-fold with water, filter (Chromatodisc 25A, Kurabou, Osaka), inject a 50-200 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Capcell Pak C18 SG-120 (Shiseido)
Mobile phase: MeOH:35 mM pH 8 phosphate buffer 30:70, containing 75 mM hydrogen peroxide and 20 mM 18-crown-6
Flow rate: 0.6
Injection volume: 50-200
Detector: F ex 325 em 448 following post-column reaction. The column effluent flowed through a 3 m × 0.25 mm ID Tefzel coil irradiated by two 4 W germicidal lamps. The effluent from this coil flowed through a 50 cm × 0.13 mm ID PEEK coil and a 2 m × 0.25 mm ID PTFE coil to the detector.

CHROMATOGRAM

Retention time: 18
Limit of detection: 400 ng/mL

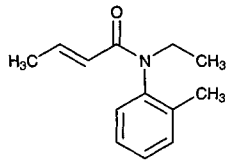
KEY WORDS

post-column reaction; post-column photochemical derivatization

REFERENCE

Mawatari,K.-i.; Mashiko,S.; Sate,Y.; Usui,Y.; Iinuma,F.; Watanabe,M. Determination of disodium cromoglycate in human urine by high-performance liquid chromatography with post-column photoirradiation-fluorescence detection, *Analyst*, **1997**, *122*, 715–717.

Crotamiton



Molecular formula: $C_{13}H_{17}NO$
Molecular weight: 203.28
CAS Registry No.: 483-63-6
Merck Index: 2661

SAMPLE

Matrix: blood, urine
Sample preparation: Evaporate 40 µL 61 µM IS in MeOH into a tube with a stream of nitrogen, add 1 mL plasma or 150 µL urine, add 1 mL pH 12 buffer (Titrisol, Merck), add 4 mL n-heptane:isoamyl alcohol 99:1, shake for 20 min at 300 rpm, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 µL mobile phase, inject a 75 (plasma) or 65 (urine) µL aliquot.

HPLC VARIABLES

Column: 200 × 4.6 5 µm Hypersil ODS
Mobile phase: MeCN:10 mM KH_2PO_4 45:55
Flow rate: 1.5
Injection volume: 65-75
Detector: UV 220

CHROMATOGRAM

Retention time: 6 (trans), 7 (cis)
Internal standard: N-ethyl-N-propionyl-o-toluidine (5)
Limit of quantitation: 320 nM (urine), 43.3 nM (plasma)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Sioufi,A.; Sandrenan,N.; Dubois,J.P. Determination of crotamiton in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *494*, 361–367.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve in THF, inject a 5 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 3 µm Spherisorb CN
Column: 250 × 2 3 µm Spherisorb CN
Mobile phase: carbon dioxide and MeOH
Column temperature: 100
Flow rate: 1.9 (carbon dioxide), 0.05 (MeOH)
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 1.9 (cis), 3.3 (trans)

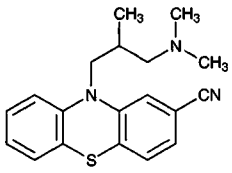
OTHER SUBSTANCES

Simultaneous: impurities, excipients

KEY WORDS
cream; SFC; 125 bar

REFERENCE
Anton,K.; Bach,M.; Geiser,A. Supercritical fluid chromatography in the routine stability control of antipruritic preparations, *J.Chromatogr.*, **1991**, *553*, 71–79.

Cyamemazine



Molecular formula: $C_{19}H_{21}N_3S$
Molecular weight: 323.46
CAS Registry No.: 3546-03-0
Merck Index: 2753

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 269

CHROMATOGRAM

Retention time: 6.71

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 270

CHROMATOGRAM

Retention time: 14.993

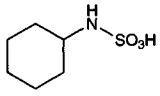
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cyclamic acid



Molecular formula: $C_6H_{13}NO_3S$
Molecular weight: 179.24
CAS Registry No.: 100-88-9, 139-05-9 (sodium salt), 139-06-0 (calcium salt)
Merck Index: 2770

SAMPLE

Matrix: food

Sample preparation: Fruit juice. Centrifuge at 3000 g for 20 min, filter (0.45 μm) the supernatant. Remove a 2 mL aliquot of the filtrate and add it to 1 mL 30% hydrogen peroxide and 300 μL 37% HCl, heat at 100° under a reflux condenser for 1 h, cool to room temperature, neutralize with 400 μL 40% NaOH, make up to 25 mL with pH 10 borate buffer. Remove a 1 mL aliquot and make up to 10 mL with reagent, after 5 min inject an aliquot. Marmalade, preserves. Stir 10 g marmalade or preserve with 50 mL water for 30 min, centrifuge at 3000 g for 20 min, remove the supernatant, suspend the residue in 10 mL water, centrifuge. Combine the supernatants and make up to 100 mL with water. Remove a 2 mL aliquot and add it to 1 mL 30% hydrogen peroxide and 300 μL 37% HCl, heat at 100° under a reflux condenser for 1 h, cool to room temperature, neutralize with 400 μL 40% NaOH, make up to 25 mL with pH 10 borate buffer. Remove a 1 mL aliquot and make up to 10 mL with reagent, after 5 min inject an aliquot. (Prepare borate buffer by dissolving 25 g boric acid in 900 mL water, adjust pH to 10 with KOH. Prepare reagent by dissolving 200 mg o-phthaldialdehyde in 5 mL EtOH, add 1 mL 3-mercaptopropionic acid, make up to 100 mL with borate buffer.)

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrospher 100 RP-18
Mobile phase: MeCN:buffer 64:36 (Prepare buffer by dissolving 3 g $Na_2HPO_4.12H_2O$ and 3 g $NaH_2PO_4.H_2O$ in 1 L water.)
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 440-650

CHROMATOGRAM

Retention time: 12
Limit of detection: 500 ng/g

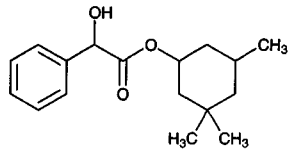
KEY WORDS

derivatization; fruit juice; marmalade; preserves

REFERENCE

Rüter,J.; Raczek,D.I.U. Empfindliches und selektives HPLC-Verfahren mit prächromatographischer Derivatisierung zur Bestimmung von Cyclamat in Lebensmitteln [Sensitive and selective HPLC procedure with prechromatographical derivatization for the determination of cyclamate in foods], *Z.Lebensm.Unters.Forsch.*, **1992**, *194*, 520–523.

Cyclandelate



Molecular formula: $C_{17}H_{24}O_3$
Molecular weight: 276.38
CAS Registry No.: 456-59-7
Merck Index: 2771
Lednicer No.: 1 94

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 5 mL 100 mM NaOH + 10 mL chloroform, shake mechanically for 10 min, centrifuge at 600 g for 10 min. Remove the aqueous layer and add it to 1 mL 2 M HCl, shake with 10 mL diethyl ether, centrifuge at 600 g for 10 min. Remove 8 mL of the organic layer and add it to 4 mL 10 mM NaOH, shake for 15 min, centrifuge at 600 g for 10 min. Remove 4 mL of the aqueous layer and add it to 500 µL 1 M HCl, add 2 mL reagent, add 300 µL hexane, shake, centrifuge at 600 g for 10 min, inject a 100 µL aliquot of the hexane layer. (Reagent was 36.8 g $Ce(NH_4)_4(SO_4)_4.2H_2O$ in 1 L 1 M sulfuric acid, stir for 30 min to dissolve, allow to stand overnight, filter.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Zorbax CN
Mobile phase: Hexane:propanol 1000:1
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 4.4 (benzaldehyde, oxidation product of the major metabolite, mandelic acid)
Limit of detection: 100 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kojima,K.; Uezono,Y.; Takahashi,T.; Nakanishi,Y. High-performance liquid chromatographic method for the determination of a cyclandelate metabolite, mandelic acid, in human plasma, *J.Chromatogr.*, **1988**, *425*, 203–207.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 26.383

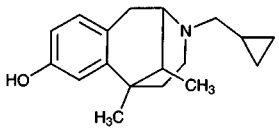
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cyclazocine



Molecular formula: $C_{18}H_{25}NO$
Molecular weight: 271.40
CAS Registry No.: 3572-80-3
Merck Index: 2773
Lednicer No.: 1 298

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

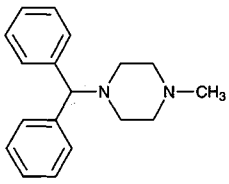
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phe-

nobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cyclizine



Molecular formula: $C_{18}H_{22}N_2$
Molecular weight: 266.39
CAS Registry No.: 82-92-8, 303-25-3 (HCl), 5897-19-8 (lactate)
Merck Index: 2779
Lednicer No.: 1 58

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 6.49
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 5 mL MeCN and 5 mL water. 1 mL Serum or 500 μL urine + 1 mL MeCN, vortex for 30 s, centrifuge at 1600 g for 5 min. Remove the supernatant and add it to 4 mL water and 100 μL 1 μg/mL IS, vortex for 30 s, add to the SPE cartridge, wash with 20 mL water, wash with 5 mL MeCN:water 70:30, dry under vacuum, elute with three 500 μL aliquots of MeCN:50 mM pH 3 sodium phosphate buffer 70:30. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 20 μL water, vortex for 1 min, add 30 μL MeCN, vortex for 1 min, centrifuge at 1600 g for 30 s, inject a 5-15 μL aliquot of the clean supernatant.

HPLC VARIABLES

Guard column: Uptight Precolumn packed with glass beads (Upchurch)

Column: 150 × 4.1 5 μm Techsil C18 (HPLC Technology)

Mobile phase: MeCN:50 mM pH 3 phosphate buffer 30:70 (Buffer was 3.2 mL/L phosphoric acid in water adjusted to pH 3.0 with solid NaOH.)

Column temperature: 30

Flow rate: 1

Injection volume: 5-15

Detector: E, Environmental Sciences Associates Model 5100A Coulochem, Model 5010 analytical cell preceded by a carbon filter, first electrode 0.55 V, second electrode 0.90 V, Model 5020 guard cell preceded by a carbon filter +1.00 V

CHROMATOGRAM

Retention time: 6

Internal standard: chlorcyclizine (12)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; SPE

REFERENCE

Walker,R.B.; Kanfer,I. Sensitive high-performance liquid chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine, in biological fluids using coulometric detection, *J.Chromatogr.B*, **1995**, *672*, 172–177.

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 50 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 50 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.3

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS

tablets; syrups; elixirs; injections

REFERENCE

Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE

Matrix: formulations

Sample preparation: Capsules, tablets. Remove contents of capsules and powder tablets. Weigh out an amount equivalent to about 10 mg loperamide hydrochloride, add 80 mL chloroform, shake for 15 min, make up to 100 mL with chloroform, filter and discard first 10-20 mL of filtrate. 5 mL Filtrate + 1 mL 400 μg/mL cyclizine hydrochloride in chloroform, make up to 25 mL with chloroform, inject an aliquot. Syrups. Add a quantity of syrup corresponding to about 10 mg loperamide hydrochloride to 30 mL water, extract four times with 20 mL portions of chloroform and filter each extract through glass wool, combine the extracts and make up to 100 mL with chloroform. Remove a 5 mL aliquot and add it to 1 mL 400 μg/mL cyclizine hydrochloride in chloroform, make up to 25 mL with chloroform, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Perkin-Elmer Analytical silica
Mobile phase: Chloroform:MeOH:ammonia 95.5:4.5:0.05
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: cyclizine

OTHER SUBSTANCES

Simultaneous: loperamide
Noninterfering: propylene glycol

KEY WORDS

capsules; tablets; syrups; normal phase; cyclizine is IS

REFERENCE

Leung,C.P.; Au-Yeung,C.Y. High-performance liquid chromatographic determination of loperamide hydrochloride in pharmaceutical preparations, *J.Chromatogr.*, **1988**, *449*, 341–344.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thiorida-

zine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 2.47

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18

Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min

Flow rate: 1.5

Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, tripelennamine, triprolidine, methaphenilene, pyrrobutamine, meclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5,

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 12.40 (A), 5.78 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

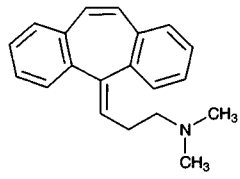
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Cyclobenzaprine



Molecular formula: $C_{20}H_{21}N$
Molecular weight: 275.39
CAS Registry No.: 303-53-7, 6202-23-9 (HCl)
Merck Index: 2782
Lednicer No.: 3 77

SAMPLE

Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL ice-cold MeOH, vortex, centrifuge at 14000 rpm for 10 min, remove the supernatant, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:0.085% phosphoric acid adjusted to pH 6.5 with triethylamine 60:40
Flow rate: 1.5
Injection volume: 80
Detector: UV 229

CHROMATOGRAM

Retention time: 18.4

OTHER SUBSTANCES

Extracted: metabolites

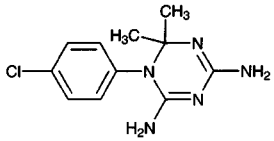
KEY WORDS

liver

REFERENCE

Wang,R.W.; Liu,L.; Cheng,H. Identification of human liver cytochrome P450 isoforms involved in the in vitro metabolism of cyclobenzaprine, *Drug Metab.Dispos.*, **1996**, *24*, 786–791.

Cycloguanil



Molecular formula: $C_{11}H_{14}ClN_5$
Molecular weight: 251.72
CAS Registry No.: 516-21-2, 609-78-9 (pamoate)
Merck Index: 2790
Lednicer No.: 1 281

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 243

CHROMATOGRAM

Retention time: 4.19
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 10.793

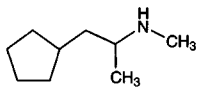
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cyclopentamine



Molecular formula: $C_9H_{19}N$
Molecular weight: 141.26
CAS Registry No.: 102-45-4, 3459-06-1 (HCl)
Merck Index: 2808

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

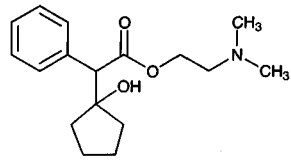
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ran-

itidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Cyclopentolate



Molecular formula: $C_{17}H_{25}NO3$
Molecular weight: 291.39
CAS Registry No.: 512-15-2, 5870-29-1 (HCl)
Merck Index: 2815
Lednicer No.: 1 92

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 75:20:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 260

KEY WORDS
chiral; α = 1.13 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

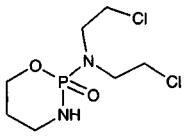
HPLC VARIABLES
Column: 150 × 2 PRP-1 (Keystone)
Mobile phase: MeCN:2-butanone:100 mM pH 7.5 phosphate buffer 40:20:40
Flow rate: 0.15
Injection volume: 1
Detector: chemiluminescence following post-column reaction. A 1 mM solution of Ru(2,2'-bipyridine)$_3^{++}$ in 50 mM sodium sulfate (continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)$_3^{+++}$ using a Princeton Applied Research Model 174A polarographic analyzer with a platinum gauze working electrode, a platinum wire auxiliary electrode, and a silver wire reference electrode. The Ru solution at 0.3 mL/min was mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.1-1 μg/mL

OTHER SUBSTANCES
Simultaneous: atropine, cyclobenzaprine, dicyclomine, procyclidine

REFERENCE
Holeman,J.A.; Danielson,N.D. Microbore liquid chromatography of tertiary amine anticholinergic pharmaceuticals with tris(2,2'-bipyridine)ruthenium(III) chemiluminescence detection, *J.Chromatogr.Sci.*, **1995**, *33*, 297–302.

Cyclophosphamide



Molecular formula: $C_7H_{15}Cl_2N_2O_2P$
Molecular weight: 261.09
CAS Registry No.: 50-18-0, 6055-19-2 (monohydrate)
Merck Index: 2816
Lednicer No.: 3 161

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak SPE cartridge with 10 mL MeOH, 10 mL air, and 10 mL water. 1 mL Serum + 15 μg IS + 5 mL water, mix, pass through the SPE cartridge, wash with 20 mL water, pass 10 mL air through the cartridge, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 100 μL water, vortex for 1 min. Add 40 μL toluene, vortex for 30 s, centrifuge at 12000 g for 1 min. Remove a 75 μL aliquot of the aqueous phase and add it to 2.5 mL chloroform, vortex for 1 min, centrifuge at 2000 g for 2 min. Remove a 2 mL aliquot of the chloroform layer, dry under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, vortex for 1 min, inject a 90 μL aliquot. (Caution: Chloroform is a carcinogen !)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18, Temp CT 38
Mobile phase: MeCN:2 mM pH 4.0 potassium phosphate buffer 29:71
Flow rate: 1.5
Injection volume: 90
Detector: UV 195

CHROMATOGRAM

Retention time: 7
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (12)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophenon, allopurinol, aspirin, carbamazepine, cisplatin, digoxin, ethosuximide, 5-fluorocytosine, 5-fluorouracil, methotrexate, phenytoin, primidone, theophylline, vincristine
Interfering: carbamazepine-10,11-epoxide, phenobarbital

KEY WORDS

serum; SPE; pharmacokinetics

REFERENCE

Hardy,R.W.; Erlichman,C.; Soldin,S.J. High-performance liquid chromatographic measurement of cyclophosphamide in serum, *Ther.Drug Monit.*, **1984**, *6*, 313–318.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Isolute CH(EC) SPE cartridge with 2 mL MeOH and 1 mL 10 mM ammonium acetate buffer. Mix 500 μL plasma with 1.5 mL 10 mM pH 4.9 ammonium acetate buffer, 50 μL 5 μg/mL IS. Add to the SPE cartridge, wash with 1 mL MeOH:10 mM ammonium acetate 10:90, elute with 300 μL MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm LiChroCART 4-4 RP-select B

Column: 250 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeOH:100 mM pH 4.9 ammonium acetate buffer 60:40
Flow rate: 1
Injection volume: 200
Detector: MS, Finnigan MAT TSQ 700, m/z 261, APCI/ESI interface, vaporizer 450°, capillary 175°, corona discharge 5 μA, argon collision gas 0.5 mtorr, collision energy -45 eV

CHROMATOGRAM
Retention time: 5
Internal standard: deuterated cyclophosphamide (5)
Limit of quantitation: 25 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Fox,P.A.; Lively,J.D.; Firth,J.W.; Woolfrey,S.G.; Greenslade,D. A sensitive assay for cyclophosphamide in human plasma utilizing mass spectrometry, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1047–1059.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg cyclohexyl SPE cartridge (Varian) with two 1 mL portions of MeOH and 1 mL water (pH 4). Add 2 mL serum to 2 mL 25 mM pH 4 phosphate buffer and 400 μL water (pH 4), vortex for 30 s, add four 1 mL portions to the SPE cartridge, let them pass through for 3-4 min, dry the cartridge using full vacuum, wash with 1 mL MeCN:water (pH 4) 10:90, elute with 1 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C8
Mobile phase: MeCN:25 mM pH 4 phosphate buffer 25:75
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Retention time: 7.6

KEY WORDS
pharmacokinetics; serum; SPE

REFERENCE
Corlett,S.A.; Chrystyn,H. High-performance liquid chromatographic determination of the enantiomers of cyclophosphamide in serum, *J.Chromatogr.B*, **1996**, *682*, 337–342.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg cyclohexyl SPE cartridge (Varian) with two 1 mL portions of MeOH and 1 mL water (pH 4). Add 2 mL serum to 2 mL 25 mM pH 4 phosphate buffer and 400 μL water (pH 4), vortex for 30 s, add four 1 mL portions of sample to the SPE cartridge, let them pass through for 3-4 min, dry the cartridge using full vacuum, wash with 1 mL MeCN:water (pH 4) 10:90, elute with 1 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot onto column A, elute with mobile phase, monitor the effluent from column A, divert the effluent from column A containing the cyclophosphamide onto column B, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 4.6 5 μm Spherisorb C1 (packed in house); B 100 × 4.0 Chiral-AGP (Chromtech, Sweden)
Mobile phase: MeCN:15 mM pH 4 phosphate buffer 1:99
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Retention time: 4.54 (R), 8.48 (S)
Limit of detection: 1250 pg/mL

KEY WORDS
chiral; pharmacokinetics; serum; SPE; column-switching; heart cut

REFERENCE
Corlett,S.A.; Chrystyn,H. High-performance liquid chromatographic determination of the enantiomers of cyclophosphamide in serum, *J.Chromatogr.B*, **1996**, *682*, 337–342.

SAMPLE
Matrix: formulations
Sample preparation: Filter (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere reverse-phase
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 2.75

OTHER SUBSTANCES
Simultaneous: ondansetron

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Fleming,R.A.; Olsen,D.J.; Savage,P.D.; Fox,J.L. Stability of ondansetron hydrochloride and cyclophosphamide in injectable solutions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 514–516.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.75
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 2.98

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Add solid NaCl to a 500 μL aliquot of the reaction mixture until some solid remains undissolved, add 250 μL MeCN, stir for 5 min, inject a 20 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:20 mM pH 4.4 KH_2PO_4 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 16.3
Limit of detection: 18000 ng/mL

OTHER SUBSTANCES
Simultaneous: ifosfamide

REFERENCE
Lunn,G.; Sansone,E.B.; Andrews,A.W.; Hellwig,L.C. Degradation and disposal of some antineoplastic drugs, *J.Pharm.Sci.*, **1989**, *78*, 652–659.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:30 mM sodium dihydrogen phosphate 23:77
Flow rate: 1
Injection volume: 20
Detector: UV 195

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Simultaneous: doxorubicin (5.4)

KEY WORDS
stability in 0.9% sodium chloride injection USP

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 30:70
Flow rate: 1.5
Injection volume: 25
Detector: UV 200

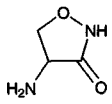
CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
buffer

REFERENCE
Kensler,T.T.; Behme,R.J.; Brooke,D. High-performance liquid chromatographic analysis of cyclophosphamide, *J.Pharm.Sci.*, **1979**, *68*, 172–174.

Cycloserine



Molecular formula: $C_3H_6N_2O_2$
Molecular weight: 102.09
CAS Registry No.: 68-41-7
Merck Index: 2820
Lednicer No.: 3 14

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 250 μL buffer + 30 μL 240 μg/mL 6-aminocaproic acid + 50 μL 6.5 mg/mL 5-methoxyindole-3-acetic acid, filter (Centriflo ultrafilter) while centrifuging at 723 g for 15 min, inject a 20-25 μL aliquot of the ultrafiltrate. Urine. 1 mL Urine + 30 μL 240 μg/mL 6-aminocaproic acid + 50 μL 3 mg/mL α-aminobutylhistidine + 1 mL 200 mg/mL sodium carbonate in water (prepare only 5-6 samples at a time), mix, add 2 mL isopropanol, vortex, centrifuge at 723 g for 5 min, inject a 25 μL aliquot of the top organic layer. (Buffer was 12.4 g boric acid + 100 mL 1 M NaOH diluted to 250 mL with water, pH 9.75.)

HPLC VARIABLES

Guard column: 30-38 μm Co:Pell ODS

Column: 240 × 5 10 μm ODS-Hypersil

Mobile phase: Isopropanol:water:glacial acetic acid:decanesulfonate 75:800:5:0.5 (plasma) or 65:800:5:0.5 (urine), pH adjusted to 4.4 with 1 M KOH

Flow rate: 2.3

Injection volume: 20-25

Detector: F ex 340 em 455 following post-column derivatization. Reagent at 1.2 mL/min is mixed with column effluent, mixture flows through a 250 × 4.5 reactor packed with 50 μm glass beads to the detector. (Reagent was o-phthalaldehyde and 2-mercaptoethanol post-column derivatizing reagent (Fluoraldehyde, Pierce).)

CHROMATOGRAM

Retention time: 5 (plasma), 6 (urine)

Internal standard: 6-aminocaproic acid (9.5), 5-methoxyindole-3-acetic acid (11 (plasma), 8 (urine), detection at UV 313), α-aminobutylhistidine (15)

Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES

Extracted: acetylacetonylcycloserine, metabolites

KEY WORDS

plasma; methoxyindoleacetic acid is IS for acetylacetonylcycloserine (UV 313 detection for both); post-column reaction

REFERENCE

Musson,D.G.; Maglietto,S.M.; Hwang,S.S.; Gravellese,D.; Bayne,W.F. Simultaneous quantification of cycloserine and its prodrug acetylacetonylcycloserine in plasma and urine by high-performance liquid chromatography using ultraviolet absorbance and fluorescence after post-column derivatization, *J.Chromatogr.*, **1987**, *414*, 121–129.

SAMPLE

Matrix: urine

Sample preparation: Filter (0.45 μm) urine. Remove a 1 mL aliquot and add a 20-fold molar excess of 2 mM 9-chloro-10-methylacridinium triflate in MeCN:pH 5.0 phosphate buffer 50:50, vortex for 10 s, heat at 70° for 30 min, add 1 mL glacial acetic acid, mix,

inject a 10 μL aliquot. (Synthesis of 9-chloro-10-methylacridinium triflate is as follows. Dissolve 6.07 g 9-chloroacridine (Eastman) in 55 mL dry dichloromethane, add 5 g methyl trifluoromethanesulfonate, stir for 3 h, filter, wash the solid with cold dichloromethane, dry in air overnight, recrystallize from MeCN to obtain 9-chloro-10-methylacridinium triflate as yellow crystals (mp 227-229°).)

HPLC VARIABLES

Column: 250 × 4.6 Partisil silica
Mobile phase: MeCN:EtOH:glacial acetic acid 50:30:20
Flow rate: 2
Injection volume: 10
Detector: F ex 257 em 475

CHROMATOGRAM

Retention time: 5.3
Limit of quantitation: 150 ng/mL

KEY WORDS

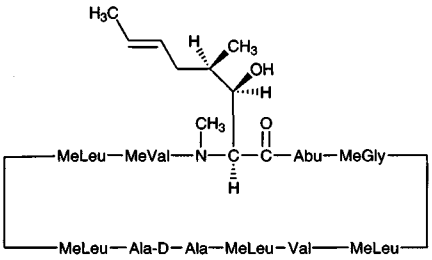
derivatization

REFERENCE

Yoo,G.S.; Choi,K.; Stewart,J.T. Second derivative ultraviolet spectrophotometry and high performance liquid chromatography with fluorometric detection of cycloserine using 9-chloro-10-methylacridinium triflate as a new UV and fluorescent labeling agent, *Anal.Lett.*, **1990**, *23*, 1245–1263.

Cyclosporine

Molecular formula: $C_{62}H_{111}N_{11}O_{12}$
Molecular weight: 1202.64
CAS Registry No.: 59865-13-3
Merck Index: 2821



SAMPLE

Matrix: blood
Sample preparation: Mix 1 ml whole blood with 100 µL 1 µg/mL cyclosporin D in EtOH, 2 mL 180 mM HCl and 7 mL diethyl ether. Shake for 5 min and centrifuge at 4200 g for 5 min. Remove the ether phase and add it to 2.5 mL 100 mM NaOH, shake and centrifuge briefly. Evaporate the ether phase to dryness under a stream of nitrogen. Reconstitute the residue with 200 µL mobile phase, inject a 200 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb S5 CN normal phase
Mobile phase: Hexane:isopropanol 90:10
Column temperature: 50
Flow rate: 1.45
Injection volume: 200
Detector: UV 212

CHROMATOGRAM

Retention time: 8.9
Internal standard: cyclosporin D (7.1)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; normal phase

REFERENCE

Khoschsorur,G.; Semmelrock,H.J.; Rödl,S.; Auer,T.; Petek,W.; Iberer,F.; Tscheliessnigg,K.H. Rapid, sensitive high-performance liquid chromatographic method for the determination of cyclosporin A and its metabolites M1, M17 and M21, *J.Chromatogr.B*, **1997**, *690*, 367–372.

SAMPLE

Matrix: microsomal incubations
Sample preparation: 500 µL Microsomal incubation + 500 µL MeCN, mix, centrifuge, inject a 100 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: ultra sepharose ODS (Beckman)
Column: 250 × 4.6 ultra sepharose ODS (Beckman)
Mobile phase: Gradient. MeCN:water from 55:45 to 60:40 over 15 min, to 70:30 over 10 min, to 90:10 over 15 min, return to initial conditions over 5 min.
Column temperature: 70
Injection volume: 100

CHROMATOGRAM
Retention time: 34.4 (cyclosporin G)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Pichard,L.; Domergue,J.; Fourtanier,G.; Koch,P.; Schran,H.F.; Maurel,P. Metabolism of the new immunosuppressor cyclosporin G by human liver cytochromes P450, *Biochem.Pharmacol.*, **1996**, *51*, 591–598.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 3 μL Hypersil ODS
Mobile phase: MeCN:water:MTBE 34:59:7.2 containing 20 mM phosphoric acid and 10 mM sodium dodecyl sulfate, adjusted to pH 2.8 with concentrated NaOH solution (A) or MeCN:water:MTBE 43:52:5 containing 20 mM phosphoric acid and 10 mM dodecyl sulfate, adjusted to pH 5.0 with concentrated NaOH solution (B)
Column temperature: 80
Flow rate: 2 (A), 1.7 (B)
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 28 (A), 29.5 (B) (cyclosporin A)

OTHER SUBSTANCES
Simultaneous: cyclosporin B, cyclosporin C, cyclosporin D, cyclosporin F, cyclosporin G, cyclosporin H, cyclosporin L, cyclosporin U, isocyclosporin A, Leu^4-cyclosporin, dihydroMeBmt^1-cyclosporin

REFERENCE
Husek,A. High-performance liquid chromatographic analysis of cyclosporin A and its oral solution, *J.Chromatogr.A*, **1997**, *759*, 217–224.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb ODS-2
Mobile phase: MeCN:water 70:30
Flow rate: 2
Detector: UV 215

CHROMATOGRAM
Retention time: 10

REFERENCE
Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

SAMPLE

Matrix: tissue

Sample preparation: Freeze four 50 μL portions of scales with liquid nitrogen and grind with purified quartz sand. Vortex the mixture in two portions. Vortex each portion twice with 2 mL MeCN for 3 min, centrifuge at 3500 rpm for 5 min. Filter (0.2 μm) the supernatant, evaporate it to dryness under the stream of nitrogen. Reconstitute the residue with 5 mL MTBE. Vortex with 2 mL 100 mM NaOH for 3 min, filter (0.2 μm), and wash the organic phase with 2 mL 100 mM HCl. Evaporate the MTBE under a stream of nitrogen. Dissolve the residue in 300 μL MeCN, dilute with 900 μL water and 200 μL mobile phase A. Filter, wash filter with 500 μL mobile phase A. Inject a 1.9 mL aliquot onto column A, elute with mobile phase A. Divert the effluent having retention time 16-25 min onto column B. Wash column B with 1 mL water, dry with a stream of pure nitrogen. Elute with 1 mL MeCN in the opposite direction. Evaporate the clear eluate to dryness, reconstitute the residue in 100 μL MeCN, add 100 μL water and IS. Inject a 20 μL aliquot onto column C and elute with mobile phase B or C.

HPLC VARIABLES

Column: A 20 × 4 5 :gmm Spherisorb Alumina PC18 purification cartridge (Bischoff Analysentechnik, Germany); B PRP-1 trap cartridge (Bischoff Analysentechnik, Germany); C 4 × 4 5 μm LiChrospher 100 RP-18 + 250 × 4.6 5 μm LiChrospher 100 RP-18

Mobile phase: A MeCN:water 29:71 containing 200 mM NaOH, pH 11; B MeCN:water 71:29; C Gradient. MeCN:water from 75:25 to 85:15 over 10 min

Column temperature: 70

Flow rate: 2 (A), 1 (B, C)

Injection volume: 20

Detector: UV 212

CHROMATOGRAM

Retention time: 11.5 (B), 7.87 (C)

Internal standard: cyclosporine C (6.25 (C), 8.6 (B))

Limit of detection: 7 ng

KEY WORDS

cutaneous scale

REFERENCE

Spöttl,T.; Eibler,E.; Wiegrebe,W. ng-Determination of cyclosporine A in cutaneous scales, *Pharmazie*, **1997**, *52*, 759–762.

SAMPLE

Matrix: whole blood

Sample preparation: Mix 2 mL whole blood with 16 μL 100 μg/mL IS in MeOH and 100 mg NaF. Vortex for 15 s, add 5 mL diethyl ether, mix for 2 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 50°. Reconstitute the residue with 150 μL mobile phase and 400 μL hexane, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 200 × 4.6 7 μm Spherisorb C8

Mobile phase: MeCN:MeOH:water:isopropanol 57:18:25:1.5

Column temperature: 65

Flow rate: 1.4

Injection volume: 20

Detector: UV 208

CHROMATOGRAM

Retention time: 4.6

Internal standard: cyclosporine D (5.5)

Limit of detection: 20 ng/mL

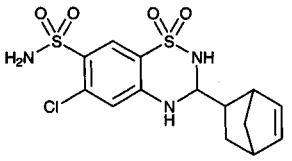
KEY WORDS

pharmacokinetics

REFERENCE

Li,K.; Wang,P.; Yuan,Y.; Liu,X. Determination of cyclosporin-A in human whole blood by reversed phase liquid chromatography with single-step extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2179–2188.

Cyclothiazide



Molecular formula: $C_{14}H_{16}ClN_3O_4S_2$
Molecular weight: 389.88
CAS Registry No.: 2259-96-3
Merck Index: 2822
Lednicer No.: 1 358

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.51

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine,

estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 13.85 (A), 14.8 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 6.6, 6.7 (two peaks)
Internal standard: 7-propyltheophylline (4.5)

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, polythiazide, probenecid, spironolactone, torsemide, triamterene
Interfering: piretanide

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18

Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 9.6

Limit of detection: 20 ng/mL.

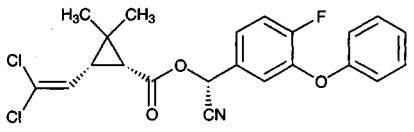
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Cyfluthrin



Molecular formula: $C_{22}H_{18}Cl_2FNO_3$
Molecular weight: 434.29
CAS Registry No.: 68359-37-5
Merck Index: 2826

SAMPLE

Matrix: formulations
Sample preparation: Weigh out sample containing 320 mg cyfluthrin, add 25 mL dioxane (Caution! Dioxane is a carcinogen!), add 10 mL 0.65% acetophenone in hexane, shake mechanically for 30 min, add 25 mL hexane, mix well. Dilute an aliquot 1:10 with hexane, mix well, filter (0.45 μm), inject a 25 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax Sil silica
Mobile phase: Hexane:dioxane 97.5:2.5
Flow rate: 1.5
Injection volume: 25
Detector: UV 230

CHROMATOGRAM

Retention time: 10.7, 11.9, 12.6, 13.7 (diastereomers)
Internal standard: acetophenone (6.7)

KEY WORDS

normal phase

REFERENCE

Slahck,S.C. Liquid chromatographic method for determination of cyfluthrin in technical and formulated products, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 595–598.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out amount of formulation containing 50 mg cyfluthrin, add 20 mL 0.2% dodecaphenone in MeCN, mix by hand (emulsifiable concentrate or flowable liquid formulation) or shake mechanically for 15 min (wettable powder), filter (0,45 μm), dilute the filtrate with MeCN or mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm cyanopropylsilane-modified silica gel
Mobile phase: MeCN:water 55:45
Flow rate: 1.5
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 10-13
Internal standard: dodecaphenone (7-9)

KEY WORDS

emulsifiable concentrate; flowable liquid; wettable powder

REFERENCE

Harbin,D.N. Quantitation of cyfluthrin in liquid and solid formulations by reversed-phase liquid chromatography: Collaborative study, *J.AOAC Int.*, **1995**, *78*, 1335–1338.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm silica (IBM)
Mobile phase: Hexane:dichloromethane:isopropanol 99:1:0.07
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.68 (trans, S), k' 9.55 (cis, R), k' 11.6 (trans, S), k' 12.9 (cis, R)

OTHER SUBSTANCES
Also analyzed: allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, permethrin, phenothrin, resmethrin, RU-11679, tetramethrin

KEY WORDS
normal phase

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

HPLC VARIABLES
Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle covalently-bonded column (Technicol)
Mobile phase: Hexane:isopropanol 99.95:0.05
Flow rate: 1
Detector: UV 230

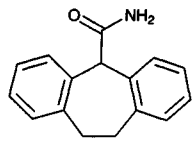
CHROMATOGRAM
Retention time: 49.0, 51.6, 59, 61.2, 64.1, 67.1, 76.0, 79.6 (enantiomers)

OTHER SUBSTANCES
Also analyzed: flucythrinate, flumethrin

KEY WORDS
chiral

REFERENCE
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

Cyheptamide



Molecular formula: $C_{16}H_{15}NO$
Molecular weight: 237.30
CAS Registry No.: 7199-29-3
Merck Index: 2828
Lednicer No.: 2 222

SAMPLE

Matrix: blood

Sample preparation: 200 μL Serum + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 14
Internal standard: cyheptamide

OTHER SUBSTANCES

Extracted: carbamazepine, carbamazepine diol, carbamazepine epoxide, 5-(p-hydroxyphenyl)-5-phenylhydantoin, lamotrigine, phenytoin
Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum; SPE; cyheptamide is IS

REFERENCE

Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

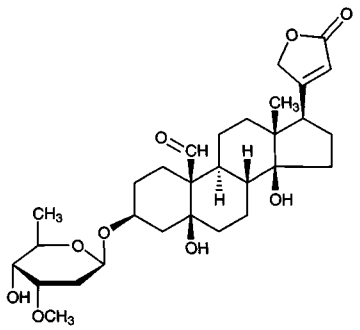
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cymarin

Molecular formula: $C_{30}H_{44}O_9$
Molecular weight: 548.67
CAS Registry No.: 508-77-0
Merck Index: 2830



SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

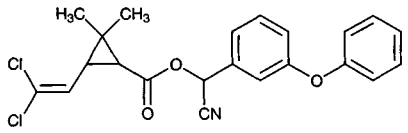
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazo-

cine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cypermethrin



Molecular formula: $C_{22}H_{19}Cl_2NO_3$
Molecular weight: 416.30
CAS Registry No.: 52315-07-8
Merck Index: 2836

SAMPLE

Matrix: bulk
Sample preparation: Dissolve the compound in benzene. Inject an aliquot. (Protect the sample from light! Caution! Benzene is a carcinogen!)

HPLC VARIABLES

Column: 250 × 4 10 μm Lichrospher Si60
Mobile phase: Hexane:benzene 50:50
Flow rate: 1
Detector: UV 280; Polarimeter, diode-laser polarimetric Chiral Monitor 2000 (Applied Chromatography System, Great Britain) collimated laser diode 30 mW at 830 nm, flow cell 4.8 cm, volume 73 μL

CHROMATOGRAM

Retention time: 4.92 (cis), 5.62 (trans) (UV detection); 3.06 (1RS, 3SR, SαCN diastereomer), 5.41 (1RS, 3SR, RαCN diastereomer), 5.74 (1RS, 3RS, SαCN), 6.10 (1RS, 3RS, RαCN) polarimetric detection)
Limit of detection: 112 μg

OTHER SUBSTANCES

Simultaneous: deltamethrin, permethrin

KEY WORDS

chiral; normal phase

REFERENCE

Díaz,A.N.; Sánchez,F.G.; Pareja,A.G. Resolution of deltamethrin, permethrin, and cypermethrin enantiomers by high-performance liquid chromatography with diode-laser polarimetric detection, *J.Chromatogr.Sci.*, **1998**, *36*, 210–216.

SAMPLE

Matrix: fruit, vegetables
Sample preparation: Prepare a cleanup column by placing 4 g Florisil, 1 g activated charcoal, and a 20 mm layer of anhydrous sodium sulfate in a 400 × 10 glass column, wash with 40 mL toluene, wash with 40 mL toluene:MeCN 99:1. Homogenize 25 g chopped fruit or vegetable with 70 mL MeOH at high speed for 3 min, filter, homogenize solid with 30 mL MeOH, filter. Combine the filtrates and add them to 60 mL toluene and 300 mL 10% NaCl in water, shake well for 3 min, let layers separate. Dry the organic layer by passing it through 20 g anhydrous sodium sulfate in a 20 mm diameter column, concentrate to about 5 mL under reduced pressure at 80°, add to the cleanup column, elute with 40 mL toluene:MeCN 99:1. Evaporate the eluate just to dryness under reduced pressure at 80°, reconstitute with 1 mL MeOH, inject an aliquot. (Reflux activated charcoal (20-40 mesh) with 1 M HCl for 4 h, wash with water until the washings are neutral, dry at 95-100° (J.Assoc.Off.Anal.Chem. 1983, 66, 1013). Heat 60-100 mesh Florisil at 200° for 24 h, cool, add 4% water, mix thoroughly, store in a sealed jar (J.Assoc.Off.Anal.Chem. 1983, 66, 1003).)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. MeCN:water from 62:38 to 78:22 over 32 min (Waters curve 6).
Column temperature: 50
Flow rate: 1.5
Detector: UV 206

CHROMATOGRAM
Retention time: 21.05-22.08 (3 peaks)
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Simultaneous: allethrin, biphenthrin, fenpropathrin, fenvalerate, flucythrinate, methothrin, permethrin, Py-115, tetramethrin

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KEY WORDS
cucumber; tomato; cabbage; apple; pear; peach; SPE

REFERENCE
Pang,G.-F.; Chao,Y.-Z.; Liu,X.-S.; Fan,C.-L. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables, *J.AOAC Int.*, **1995**, *78*, 1474–1480.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Pirkle type 1-A (Regis)
Mobile phase: Hexane:isopropanol 99.9:0.1
Flow rate: 1
Detector: UV 240 or 280

CHROMATOGRAM
Retention time: 30-55 (various isomers)

KEY WORDS
chiral

REFERENCE
Cayley,G.R.; Simpson,B.W. Separation of pyrethroid enantiomers by chiral high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *356*, 123–134.

SAMPLE
Matrix: solutions
Sample preparation: Extract cotton swabs 3-4 times with 500 mL hexane, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm Spherisorb S5W
Mobile phase: Hexane:1,4-dioxane 99.8:0.2
Flow rate: 1
Injection volume: 50
Detector: UV 233

KEY WORDS
normal phase

REFERENCE

Eadsforth,C.V.; Bragt,P.C.; van Sittert,N.J. Human dose-excretion studies with pyrethroid insecticides cypermethrin and αcypermethrin: relevance for biological monitoring, *Xenobiotica*, **1988**, *18*, 603–614.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in mobile phase.

HPLC VARIABLES

Column: 250 × 4.6 10 μm silica (Perkin-Elmer PE-0258-0051)
Mobile phase: Hexane:pentane:ether 78:18.1:3.9
Column temperature: 25
Flow rate: 1.5
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 7.5, 8.5, 9.5, 11 (geometrical isomers)

REFERENCE

Wang,Q-S.; Gao,R.-Y.; Wang,H.-Y. Computer-assisted optimization of selectivity (mobile phase, pH, and ion concentration) in high-performance liquid chromatography, *J.High Res.Chromatogr.*, **1990**, *13*, 173–177.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

HPLC VARIABLES

Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.85:0.15
Flow rate: 1.3
Detector: UV 230

CHROMATOGRAM

Retention time: 21.4, 23.1, 25.9, 27.8, 29.0, 34.4, 35.3 (enantiomers)

OTHER SUBSTANCES

Also analyzed: allethrin, fenpropathrin, fenvalerate, tetramethrin

KEY WORDS

chiral

REFERENCE

Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Cyclobond I cyclodextrin-modified silica (Astec)
Mobile phase: MeCN:water 225:27
Flow rate: 1

Detector: UV 220

CHROMATOGRAM
Retention time: 11, 12, 13, 17, 19 (isomers)

KEY WORDS
comparison with GC

REFERENCE
Kutter,J.P.; Class,T.J. Diastereoselective and enantioselective chromatography of the pyrethroid insecticides allethrin and cypermethrin, *Chromatographia*, **1992**, *33*, 103–112.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 0.06 μL aliquot of a 1-250 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 250 × 0.5 Spherisorb ODS2 PEEK column
Mobile phase: Isopropanol:water 70:30 containing 10 mM ammonium acetate and 22 mM formic acid
Flow rate: 0.01
Injection volume: 0.06
Detector: MS, VG Quattro tandem quadrupole, electrospray, high voltage lens 550 V, sampling cone 40-120 V, collision gas argon, collision energy 25 eV, mobile phase at 5 μL/min was used as make-up flow, m/z 433

CHROMATOGRAM
Retention time: 9
Limit of detection: 60 pg (SIM)

KEY WORDS
microbore

REFERENCE
Fleet,I.A.; Monaghan,J.J.; Gordon,D.B.; Lord,G.A. Microbore liquid chromatography-electrospray mass spectrometry of selected synthetic pyrethroid insecticides, *Analyst*, **1996**, *121*, 55–59.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 mL river water on to column A at 5 mL/min and let the effluent flow to waste, backflush the contents of column A on to column B and start the gradient, monitor the effluent from column B. At the end of each run backflush column A with 5 mL water, with 30 mL 100 mM pH 2 sodium citrate buffer, with 10 mL water, with 5 mL MeCN, and with 10 mL hexane:dichloromethane 50:50. Wash new column A with 10 mL water.

HPLC VARIABLES
Column: A 20 × 3 10 μm PRP-1 (Hamilton); B 30 × 4.6 10 μm RP-18 (Brownlee) + 250 × 4.6 5 μm LiChrospher C18
Mobile phase: Gradient. MeCN:water from 5:95 to 90:10 over 1 h, maintain at 90:10 for 4 min, return to initial conditions (?) over 1 min, re-equilibrate for 10 min.
Flow rate: 1 for 64 min, to 1.5 over 1 min, maintain at 1.5 for 5 min, to 1 over 5 min
Injection volume: 100000
Detector: UV 210

CHROMATOGRAM
Retention time: 59.8

Limit of detection: 100 ng/L

OTHER SUBSTANCES

Simultaneous: alachlor, aldicarb, aldicarb oxime, atrazine, carbofuran, chlorobenzilate, chlorothalonil, chlorpyrifos methyl, chlortoluron, p,p'-DDE, DDT, deltamethrin, diazinon, diclofop methyl, dimethoate, diuron, ethofumesate, fenitrothion, fenvalerate, fluazifop butyl, fluometuron, linuron, metalaxyl, metamitron, methomyl, metobromuron, metolachlor, molinate, oxamyl, paraoxon, paraoxon methyl, parathion, parathion methyl, pendimethalin, permethrin, phenmediphan, pirimphos, pirimphos methyl, prometryne, propanil, propiconazole, simazine, terbuthylazine, trifluraline

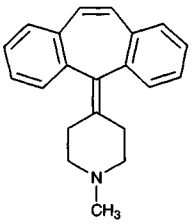
KEY WORDS

river water; column-switching

REFERENCE

Papadopoulou-Mourkidou,E.; Patsias,J. Development of a semi-automated high-performance liquid chromatographic-diode array detection system for screening pesticides at trace levels in aquatic systems of the Axios River basin, *J.Chromatogr.A*, **1996**, *726*, 99–113.

Cyproheptadine



Molecular formula: $C_{21}H_{21}N$
Molecular weight: 287.40
CAS Registry No.: 129-03-3, 41354-29-4 (HCl sesquihydrate), 969-33-5 (HCl)
Merck Index: 2842
Lednicer No.: 1 151

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 50 μL MeOH:100 mM HCl 50:50 + 50 μL 1 μg/mL desmethyldoxepin hydrochloride in 100 mM HCl + 100 μL 1.5 M NaOH + 3 mL hexane:isoamyl alcohol 99:1, vortex for 2 min, freeze, thaw, centrifuge at 2000 g for 5 min. Remove the organic layer, repeat the extraction. Combine the organic layers and add them to 100 μL 50 mM sulfuric acid, vortex for 2 min, inject a 90 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:100 mM pH 4.7 phosphate buffer containing 5 mM pentanesulfonic acid 41:15:44
Flow rate: 1.5
Injection volume: 90
Detector: UV 228

CHROMATOGRAM

Retention time: 9.40
Internal standard: desmethyldoxepin (5.19)
Limit of quantitation: 3 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat

REFERENCE

Novak,E.A.; Stanley,M.; McIntyre,I.M.; Hryhorczuk,L.M. High-performance liquid chromatographic method for quantification of cyproheptadine in serum or plasma, *J.Chromatogr.*, **1985**, *339*, 457–461.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30

Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 7.68
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver

homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 30.43
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexiphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 224

CHROMATOGRAM
Retention time: 15.015

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in water.

HPLC VARIABLES
Column: 250 × 4.6 Rexchrome ODS
Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 25:20:55
Flow rate: 2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 8
Internal standard: cyproheptadine

OTHER SUBSTANCES
Simultaneous: diltiazem

KEY WORDS
tablets; cyproheptadine is IS

REFERENCE
Shivram,K.; Shah,A.C.; Newalkar,B.L.; Kamath,B.V. Stability indicating high-performance liquid chromatographic method for the assay of diltiazem hydrochloride in tablets, *J.Liq.Chromatogr.*, **1992**, *15*, 2417–2422.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize tissue with four volumes of water. 2 mL Homogenate + 2 mL 300 mM pH 3.0 citric phosphate buffer, mix, wash with 20 mL heptane, add 2 mL 2 M NaOH, extract with 15 mL ether. Remove the organic layer and wash it with 5 mL 50 mM pH 10.0 carbonate buffer. Add the organic layer to 1 mL pH 3.0 100 mM citric phosphate buffer, mix. Remove the aqueous layer and add it to 2.5 mL 1 M NaOH, extract with 5 mL chloroform. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot. (Use chloroform with EtOH as a preservative.)

HPLC VARIABLES

Column: Partisil 10/25 ODS

Mobile phase: MeOH:30 mM KH_2PO_4 60:40
Flow rate: 1.4
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 12.5
Internal standard: diphenylpyraline.HCl (4-diphenylmethoxy-1-methylpiperidine (9.5)
Limit of detection: 500 ng/g

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; pancreas; liver; lung; kidney; brain

REFERENCE
Chow,S.A.; Fischer,L.J. Metabolism and disposition of cyproheptadine and desmethylcyproheptadine in pregnant and fetal rats, *Drug Metab.Dispos.*, **1987**, *15*, 740–748.

SAMPLE
Matrix: urine
Sample preparation: Condition a 3 mL Suplelclean C18 SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Urine + 100-120 μL MeOH, mix thoroughly, add to the SPE cartridge, wash with two 2 mL portions of MeOH:water 80:20, elute with 6 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL MeOH, inject a 20 μL aliquot.

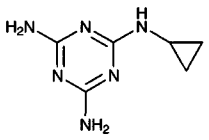
HPLC VARIABLES
Column: 150 × 6 octyl ODP
Mobile phase: MeOH:acetate buffer (ionic strength 0.005 I) 44:56 adjusted to pH 3.6 with acetic acid
Column temperature: 40
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.30
Limit of detection: 15 ng/mL
Limit of quantitation: 50 ng/mL

KEY WORDS
SPE

REFERENCE
Kountourellis,J.E.; Ebete,K.O. Reversed-phase high performance liquid chromatographic determination of cyprohepadine from urine by solid-phase extraction, *J.Chromatogr.B*, **1995**, *664*, 468–471.

Cyromazine



Molecular formula: $C_6H_{10}N_6$
Molecular weight: 166.19
CAS Registry No.: 66215-27-8
Merck Index: 2845

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM

Retention time: 3.283

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 10 μm Hibar RP-8
Mobile phase: MeCN:0.5 mM sulfuric acid 50:50
Flow rate: 1
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 6.89
Limit of detection: 20 ppb

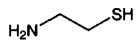
OTHER SUBSTANCES

Simultaneous: melamine, metabolites

REFERENCE

Cabras,P.; Meloni,M.; Spanedda,L. High-performance liquid chromatographic separation of cyromazine and its metabolite melamine, *J.Chromatogr.*, **1990**, *505*, 413–416.

Cysteamine



Molecular formula: C_2H_7NS
Molecular weight: 77.15
CAS Registry No.: 60-23-1
Merck Index: 2848

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 500 μL 7 M pH 9.0 urea + 1 drop of 1-octanol + 50 μL 100 mg/mL sodium borohydride in 100 mM NaOH, heat at 50° for 30 min, add 500 μL cold 10% trichloroacetic acid, centrifuge for 5 min, filter (0.45 μm), pass nitrogen through filtrate for several min to exclude oxygen, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm BAS biophase ODS (Bioanalytical Systems)
Mobile phase: 50 mM Chloroacetic acid containing 3 mL/L 70% ethylamine and 60 mg/L sodium octyl sulfate, adjust the pH to 3.0 with solid monochloroacetic acid.
Flow rate: 1
Injection volume: 20
Detector: E, Bioanalytical Systems, gold/mercury electrode +0.15 V

CHROMATOGRAM

Retention time: 8
Limit of detection: 50 nm

OTHER SUBSTANCES

Extracted: cysteine, homocysteine, glutathione

KEY WORDS

plasma; use stainless steel tubing between pump and reservoir; continuously purge mobile phase with nitrogen; cysteamine is the sum of cysteamine, cystamine, cysteine-cysteamine disulfide, and other cysteamine disulfides; pharmacokinetics

REFERENCE

Smolin,L.A.; Schneider,J.A. Measurement of total plasma cysteamine using high-performance liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1988**, *168*, 374–379.

SAMPLE

Matrix: blood
Sample preparation: 150 μL Plasma + 15 μL 100 mL/L tri-n-butylphosphine in DMF, mix, let stand at 4° for 30 min, add 150 μL 100 g/L trichloroacetic acid, mix, centrifuge. Remove a 50 μL aliquot of the supernatant and add it to 10 μL 1.55 M NaOH, add 125 μL 4 mM EDTA in 125 mM pH 9.5 borate buffer, add 50 μL 1 g/L 7-fluoro-2,1,3-benzoxadiazole-4-sulfonic acid, ammonium salt (Fluka), heat at 60° for 1 h, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 4.6 mm ID 5 μm Spherisorb ODS
Mobile phase: MeCN:100 mM pH 2.0 KH_2PO_4 4:96
Flow rate: 0.8
Injection volume: 20
Detector: F (wavelengths not given)

CHROMATOGRAM

Retention time: 5

Internal standard: cysteamine

OTHER SUBSTANCES

Extracted: cysteine, cysteinyl glycine, glutathione, homocysteine

KEY WORDS

derivatization; plasma; cysteamine is IS

REFERENCE

Kuo,K.; Still,R.; Cale,S.; McDowell,I. Standardization (external and internal) of HPLC assay for plasma homocysteine, *Clin.Chem.*, **1997**, *43*, 1653–1655.

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize (Potter-Elvehjem PTFE-glass homogenizer) tissue in 20 mM EDTA, adjust to 1% (w/v) (kidney) or 2.5% (w/v) (spleen). Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Plasma. Dilute rat plasma to 20% (v/v) with 20 mM EDTA. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Serum. Dilute human serum to 10% (v/v) with 20 mM EDTA. 1 mL Diluted serum + 200 μL 30% metaphosphoric acid, centrifuge at 2000 g at 4° for 20 min. Remove 500 μL of the supernatant and add it to 240 μL 2 M KOH. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. (Synthesis of N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide is as follows. Add 8.8 g aluminum trichloride to 12.50 g 3-dimethylaminophenol in 185 mL chloroform and 84 g triethyl orthoformate, mix at room temperature for 10 min, when the exothermic reaction ceases add 50 mL 10% HCl, stir to hydrolyze the acetal, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform, wash the filtrate with saturated aqueous NaCl, dry over magnesium sulfate, concentrate under reduced pressure, recrystallize from chloroform to give 4-(dimethylamino)salicylaldehyde (mp 78-79°). Add 400 mg KOH in 3 mL EtOH to a solution of 1 g 4-(dimethylamino)salicylaldehyde and 1.3 g (?) 4-nitrobenzylbromide in 12 mL EtOH, reflux for 7 h, cool, filter to recover the crystals, wash with water, dry under vacuum, recrystallize from EtOH to give 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde (mp 179-180°). Add a solution of 900 mg 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde in 6 mL DMF to a sodium methoxide solution (prepared from 69 mg sodium in 1 mL MeOH), reflux for 20 min, add 1 mL MeOH, filter the crystals, recrystallize from EtOH to give 6-dimethylamino-2-(4-nitrophenyl)benzofuran as red needles (mp 209.5-210.5°). Reflux 1 g 6-dimethylamino-2-(4-nitrophenyl)benzofuran in 20 mL benzene (Caution! Benzene is a carcinogen!) and 18 mL MeOH containing 80 mg active carbon and a catalytic amount of ferric chloride hexahydrate for 10 min, add 2.30 g 98% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) dropwise, reflux for 7 h, filter, concentrate the filtrate, recrystallize from cyclohexane to give 6-dimethylamino-2-(4-aminophenyl)benzofuran as orange needles (mp 198.5-200°). Stir 605 mg 6-dimethylamino-2-(4-aminophenyl)benzofuran and 230 mg maleic anhydride in 5 mL chloroform at room temperature for 3 h, filter the crystals, wash with a small amount of chloroform, recrystallize from EtOH to obtain N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid (mp 219.5-221°). Reflux a mixture of 1.17 g N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid and 30 mg sodium acetate in 18 mL acetic anhydride, cool in an ice bath, collect the crystals of product, wash with water. Neutralize the filtrate with 20% NaOH, extract twice with 30 mL portions of chlo-

roform, wash the organic layers with saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to give more product. Combine the products and recrystallize them from acetone to give N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide as reddish purple crystals (mp 203-204°) (Bull.Chem.Soc.Jpn. 1985, 58, 2192).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Toyo Soda ODS-80
Mobile phase: MeCN:10 mM pH 7.7 phosphate buffer 50:50 containing 30 mM tetrabutylammonium bromide
Flow rate: 0.8
Injection volume: 20
Detector: F ex 355 em 457

CHROMATOGRAM
Retention time: 10
Internal standard: disodium 6-amino-1,3-naphthalene disulfonate (3.5)
Limit of detection: 50 fmole

OTHER SUBSTANCES
Extracted: homocysteine, reduced glutathione (GSH), N-acetylcysteine, cysteine, coenzyme A

KEY WORDS
plasma; serum; rat; human; liver; kidney; spleen; derivatization

REFERENCE
Nakashima,K.; Umekawa,C.; Yoshida,H.; Nakatsuji,S.; Akiyama,S. High-performance liquid chromatography-fluorometry for the determination of thiols in biological samples using N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]-maleimide, *J.Chromatogr.*, **1987**, *414*, 11–17.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL Solution + 300 μL reagent solution, let stand at room temperature for 20 min, add 500 μL 300 mM phosphoric acid solution, make up to 10 mL with water, inject a 50 μL aliquot. (Prepare the reagent solution by dissolving 3.5 mg methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate in 10 mL THF, make up to 25 mL with pH 7.5 borate buffer. Prepare methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as follows. Dissolve 5 g 6'-methoxy-2'-acetonaphthone in warm glacial acetic acid and add 2.5 g glyoxylic acid, reflux for 24 h, evaporate to dryness under reduced pressure. Take up the residue in chloroform and extract it three times with 5% sodium carbonate solution. Combine the aqueous layers and acidify them with concentrated HCl, collect the product by filtration, recrystallize from MeOH/water or acetic acid to give 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid (mp 167-9°) (Farmaco, Ed. Sci. 1982, 37, 171). Reflux 0.5 g 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid, 2.5 mL MeOH, and 2-3 drops sulfuric acid in 25 mL anhydrous benzene (Caution! Benzene is a carcinogen!) for 1 h, add 20 mL water, wash the organic layer with 10 mL 5% sodium bicarbonate solution, wash the organic layer with 20 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, purify by flash chromatography on silica gel using ethyl acetate:light petroleum (bp 40-70°) 40:60 to give methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as a pale yellow compound (mp 116-120°).)

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb RP-8
Mobile phase: MeOH:50 mM pH 3.0 triethylammonium phosphate 53:47
Flow rate: 1
Injection volume: 50
Detector: F ex 310 em 450

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: acetylcysteine, cysteine, glutathione, homocysteine, mesna
Noninterfering: bacitracin, biotin, calcium pantothenate, cystine, glycine, magnesium oxide, neomycin, starch, threonine, vitamin E, pyridoxine, riboflavin phosphate

KEY WORDS
solutions

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P.; Pinzauti,S. High-performance liquid chromatographic determination of aliphatic thiols with aroylacrylic acids as fluorogenic precolumn derivatization reagents, *J.Chromatogr.*, **1990**, *507*, 451–458.

SAMPLE
Matrix: tissue
Sample preparation: 10 g Tissue + 40 mL mobile phase + 500 μL 5 mg/mL dithiothreitol in mobile phase, homogenize (VirTis Model 45 with Turbo-Shear blades (No. 16-107)) at medium setting for 2 min, centrifuge at 0-5° at 5000 rpm for 30 min, filter through glass wool, filter (Centrex 0.2 μm nylon) while centrifuging at 0-5° at 3000 rpm for 20 min, inject a 15 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil PXS 10/25 SCX
Mobile phase: MeCN:buffer 50:50 (Buffer was 200 mM formic acid, 100 mM KOH, and 0.2 mM EDTA, pH 3.5.)
Flow rate: 1.1
Injection volume: 15
Detector: E, Bioanalytical Systems LC-4B, Au/Hg electrode + 0.15 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 100 ppb

KEY WORDS
pig; muscle

REFERENCE
Pensabene,J.W.; Doerr,R.C.; Fiddler,W. Determination by liquid chromatography with electrochemical detection of cysteamine and cysteine, possible precursors of N-nitrosothiazolidine, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 1033–1035.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize tissue in ice-cold 6% 5-sulfosalicylic acid to give a 40% tissue homogenate, centrifuge at 10000 g for 10 min. Extract the supernatant with two volumes of ice-cold chloroform:MeOH 50:50, centrifuge. Remove the aqueous layer and evaporate it to the original volume under a stream of nitrogen, adjust the pH to 5.7-6.3 with 10 M KOH, add one volume of tributylphosphine:propanol 10:90 for each 31 volumes of aqueous solution, shake gently for 1 h under a gentle nitrogen flow, adjust pH to 3 with 4% 5-sulfosalicylic acid, dilute with at least an equal volume of buffer, inject a 100 μL aliquot. (Buffer was 25 mM pH 3 monochloroacetate containing 0.5 mM sodium octyl sulfate and 0.015% EDTA.)

HPLC VARIABLES
Guard column: μBondapak C18
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeOH:buffer 2:98 (Buffer was 25 mM pH 3 monochloroacetate containing 0.5 mM sodium octyl sulfate and 0.015% EDTA.)
Flow rate: 1
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, gold/mercury amalgam electrode +0.2 V

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 100 nM

OTHER SUBSTANCES
Extracted: cysteine, cystine

KEY WORDS
rat; pig; cow; liver; kidney; heart

REFERENCE
Garcia,R.A.G.; Hirschberger,L.L.; Stipanuk,M.H. Measurement of cyst(e)amine in physiological samples by high performance liquid chromatography, *Anal.Biochem.*, **1988**, *170*, 432–440.

SAMPLE
Matrix: urine
Sample preparation: Add 10 μL 50 μm 2-mercaptoethanol and 100 μL 10% trichloroacetic acid containing 10 mM EDTA to 100 μL urine, centrifuge at 760 g at 4° for 10 min. Add 350 μL 1 M pH 10.5 potassium borate buffer, 100 μL 1% tri-n-butylphosphine in water, and 100 μL 0.3% ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate in water to a 150 μL aliquot of the supernatant yielding a final pH of about 8.5. Incubate the mixture at 60° for 60 min, then put in an ice bath and add 50 μL 4 M HCl, inject a 10 μL aliquot of this solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil 5C-18AR (Nakarai Tesque, Japan)
Mobile phase: MeOH:75 mM pH 2.9 sodium citrate buffer 2:98
Flow rate: 1
Injection volume: 10
Detector: F ex 386 em 516

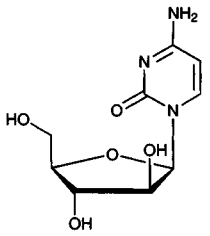
CHROMATOGRAM
Retention time: 6.0
Internal standard: 2-mercaptoethanol

OTHER SUBSTANCES
Simultaneous: acetylcysteine, cysteine, cysteinylglycine, γ-glutamylcysteine, glutathione, homocysteine

KEY WORDS
derivatization; mouse

REFERENCE
Oe,T.; Ohyagi,T.; Naganuma,A. Determination of γ-glutamylglutathione and other low-molecular-mass biological thiol compounds by isocratic high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1998**, *708*, 285–289.

Cytarabine



Molecular formula: $C_9H_{13}N_3O_5$
Molecular weight: 243.22
CAS Registry No.: 147-94-4, 69-74-9 (HCl)
Merck Index: 2853

SAMPLE

Matrix: blood

Sample preparation: Isolate mononuclear cells from 10 mL blood by a standard step-gradient density centrifugation procedure. Wash once with PBS, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash the pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, neutralize with 10 M KOH, bring to pH 7 with 1 M KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject a 50-2000 μL aliquot of the supernatant. (PBS was 8.1 g NaCl, 0.22 g KCl, 1.14 g $NaHPO_4$ (sic), 0.27 g KH_2PO_4 in 1 L water, pH 7.4.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil 10 SAX

Mobile phase: Gradient. A was 5 mM pH 2.8 $(NH_4)H_2PO_4$. B was 750 mM pH 3.5 $(NH_4)H_2PO_4$. A:B from 70:30 to 0:100 over 30 min (concave gradient, Waters no. 9). (At the start of each day pump through 20 mL 2 M $(NH_4)H_2PO_4$ then inject 100 μL 100 mM disodium EDTA into the initial mobile phase.)

Flow rate: 3

Injection volume: 50-2000

Detector: UV 262

CHROMATOGRAM

Retention time: 21 (as triphosphate, ara-CTP)

OTHER SUBSTANCES

Extracted: F-araATP (fludarabine triphosphate), ATP, CTP, UTP, GTP

KEY WORDS

mononuclear cells

REFERENCE

Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

SAMPLE

Matrix: blood

Sample preparation: Add tetrahydrouridine to plasma to a final concentration of 200 μM, filter (Amicon Centricon-10 cut-off 10 000) while centrifuging at 4° at 5000 g for 30 min. Mix 50 μL ultrafiltrate with 10 μL 20 μg/mL adenosine arabinoside, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Hypersil ODS

Mobile phase: MeOH:buffer 5:95 (Buffer was 50 mM pH 3.0 phosphate containing 0.4 mM sodium 1-heptanesulfonate.)

Column temperature: 40

Flow rate: 1
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 5.9
Internal standard: adenosine arabinoside (ara-A, vidarabine) (9.4)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: uracil arabinoside
Noninterfering: allopurinol, cephalosporins, ciprofloxacin, diazepam, metoclopramide, mitoxantrone, ondansetron

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Burk,M.; Volmer,M.; Fartash,K.; Schneider,W. Ion-pair liquid chromatography of cytarabine and uracil-arabinoside in human plasma, *Arzneimittelforschung*, **1995**, *45*, 616–619.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 278

CHROMATOGRAM
Retention time: 3.10
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazo-

cine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: Add tetrahydrouridine (deaminase inhibitor) to blood at a concentration of 0.1 mM, centrifuge at 800 g for 10 min, collect plasma. Add tetrahydrouridine (deaminase inhibitor) to CSF at a concentration of 0.1 mM. 0.5 mL Plasma or CSF + 0.5 μg deoxyinosine, mix, filter (Amicon MPS-1 micropartition, cut-off 10000) at 1000 g for 30 min, inject 100 μL of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:3,5 mM pH 3.3 KH_2PO_4 2:98
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 13.0
Internal standard: deoxyinosine (30.0) (UV 264)
Limit of detection: 5 (CSF), 15 (plasma)

OTHER SUBSTANCES

Extracted: ara-U
Noninterfering: uric acid, hypoxanthine, xanthine, cytidine, deoxycytidine, uridine, deoxyuridine, MTX, folinic acid, alizapride, chlorpromazine, promazine
Interfering: oxypurinol, allopurinol

KEY WORDS

plasma; ultrafiltrate

REFERENCE
Riccardi,A.; Servidei,T.; Lasorella,A.; Riccardi,R. High-performance liquid chromatographic assay for cytosine arabinoside and uracil arabinoside in cerebrospinal fluid and plasma, *J.Chromatogr.*, **1989**, *497*, 302–307.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. 3 mg Bulk drug + 5 mL 1.4 mg/mL p-toluic acid in MeOH + 25 mL mobile phase, inject a 10 μL aliquot. Formulations. Make up vial with sterile water to give a 1 mg/mL solution. Add 3 mL of this solution to 5 mL 1.4 mg/mL p-toluic acid in MeOH and 20 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 5:95 containing 1.34 g/L Na_2HPO_4 and 0.71 g/L $NaH_2PO_4.H_2O$, apparent pH 7.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: p-toluic acid

OTHER SUBSTANCES
Simultaneous: cyclocytidine, azacitidine, uracil arabinoside, cytosine, azacytosine, metabolites

REFERENCE
Kissinger,L.D.; Stemm,N.L. Determination of the antileukemia agents cytarabine and azacitidine and their respective degradation products by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *353*, 309–318.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cell suspension at 400 g, suspend the pellet in 0.9% NaCl, centrifuge, discard the supernatant. Add 120 μL MeCN:2.5 mM PIC A low UV (Waters) 0.2:99.8 (pH 3.0) to the pellet, vortex vigorously, centrifuge, filter (5 μm)

HPLC VARIABLES
Guard column: 30 × 5 5 μm C18 (Macherey-Nagel)
Column: 250 × 4.6 5 μm C18 (Macherey-Nagel)
Mobile phase: Gradient. A was MeCN:2.5 mM PIC A low UV (Waters) 0.2:99.8, pH 3.0. B was MeCN:100 mM KH_2PO_4 containing 5 mM PIC A low UV (Waters) 0.5:99.5, pH 2.7. A:B 100:0 for 17 min, to 0:100 (step gradient), after 28 min re-equilibrate at 100:0 at 1.5 mL/min for 10 min.
Column temperature: 21
Flow rate: 0.8 for 18 min, to 1.5 over 1 min, maintain at 1.5 for 26 min
Injection volume: 500
Detector: radioactivity (calcium fluoride solid scintillator)

CHROMATOGRAM
Retention time: 5.24
Limit of detection: 40 pg

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
tritium labeled

REFERENCE
Braess,J.; Pfortner,J.; Kaufmann,C.C.; Ramsauer,B.; Unterhalt,M.; Hiddemann,W.; Schleyer,E. Detection and determination of the major metabolites of [3H]cytosine arabinoside by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *676*, 131–140.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 1.45

OTHER SUBSTANCES
Simultaneous: fluorouracil, granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot

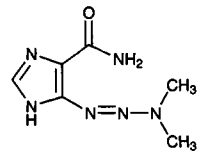
HPLC VARIABLES
Column: 250 × 5 5 μm Spherisorb 5 ODS
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 7
Flow rate: 1.3
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: cytidine, cytosine, aza-analogs

REFERENCE
Romanová,D.; Novotny,L. Chromatographic properties of cytosine, cytidine and their synthetic analogues, *J.Chromatogr.B*, **1996**, *675*, 9–15.

Dacarbazine



Molecular formula: $C_6H_{10}N_6O$
Molecular weight: 182.19
CAS Registry No.: 4342-03-4
Merck Index: 2866
Lednicer No.: 2 254

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL MeOH:chloroform 3:1, vortex for 60 s, let stand at 4° for 15 min, centrifuge at 4° at 1300 g for 10 min, inject an aliquot of the supernatant. (Caution ! Chloroform is a carcinogen !)

HPLC VARIABLES

Column: 300 × 3.9 10µm. µBondapak phenyl
Mobile phase: Gradient. MeOH:0.1% pH 5.5 ammonium formate buffer:water 5:90:5, for 0.5 min, to 30:40:30 over 1 min, maintain at 30:40:30 for 7.5 min, re-equilibrate at initial conditions for 4 min.
Flow rate: 2
Injection volume: 20–100
Detector: UV 254

CHROMATOGRAM

Retention time: 6.8
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: 5-aminoimidazole-4-carboxamide, 2-azahypoxanthine

KEY WORDS

plasma

REFERENCE

Tate,P.S.; Briele,H.A. Reversed-phase high-performance liquid chromatography of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and metabolites, *J.Chromatogr.*, **1986**, *374*, 421–424.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 325

CHROMATOGRAM

Retention time: 3.16
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 0.5-1 mL Plasma + 100 μL 100 μg/mL 3-methylxanthine, filter (Amicon CF-25), inject a 60 μL aliquot of the ultrafiltrate. Urine. 1 mL Urine + 100 μL 100 μg/mL 3-methylxanthine, dilute six-fold with water, filter (25 mm Swinnex), inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 45 × 4 10 μm Spherisorb octadecylsilyl
Column: 300 × 4 μBondapak C18

Mobile phase: Gradient. A was 500 mM sodium acetate adjusted to pH 7.0 with 10% phosphoric acid. B was MeCN:50 mM sodium acetate adjusted to pH 5.5 with concentrated phosphoric acid 25:75. A:B 100:0 for 5 min, then to 5:95 over 3 min, maintain at 5:95 for 6 min. Re-equilibrate at initial conditions for 6 min.
Injection volume: 50-60
Detector: UV 280

CHROMATOGRAM
Retention time: 12.3
Internal standard: 3-methylxanthine (11.6)
Limit of detection: 5000 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 2-azahypoxanthine, 5-aminoimidazole-4-carboxamide

KEY WORDS
plasma; protect from light; ultrafiltrate

REFERENCE
Fiore,D.; Jackson,A.J.; Didolkar,M.S.; Dandu,V.R. Simultaneous determination of dacarbazine, its photolytic degradation product, 2-azahypoxanthine, and the metabolite 5-aminoimidazole-4-carboxamide in plasma and urine by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1985**, *27*, 977–979.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 323.5

CHROMATOGRAM
Retention time: 3.6

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 220 × 4.6 5 μm silica (Brownlee)
Mobile phase: MeCN:6.25 mM NaH_2PO_4 adjusted to pH 3.0 with concentrated phosphoric acid 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 216

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Simultaneous: doxorubicin, ondansetron
Noninterfering: degradation products

KEY WORDS
injections; 5% dextrose

REFERENCE
King,D.T.; Stewart,J.T. HPLC determination of dacarbazine, doxorubicin, and ondansetron mixture in 5% dextrose injection on underivatized silica with an aqueous-organic mobile phase, *J.Liq.Chromatogr.*, **1993**, *16*, 2309–2323.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 2.32

OTHER SUBSTANCES
Simultaneous: cimetidine (UV 228), cisplatin (UV 198), granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ + 0.1% triethylamine (pH 10.0) 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 325

CHROMATOGRAM

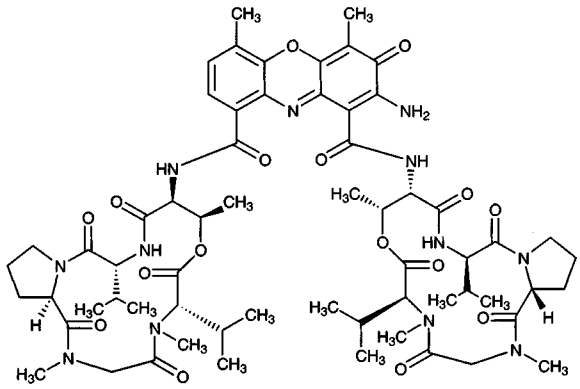
Retention time: 6.4
Limit of detection: 500 ng/mL

REFERENCE

Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Dactinomycin

Molecular formula: $C_{62}H_{86}N_{12}O_{16}$
Molecular weight: 1255.44
CAS Registry No.: 50-76-0
Merck Index: 2867



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 2 M HCl, vortex for 10 s, add 3 mL ethyl acetate saturated with water, vortex for 30 s, centrifuge at 300 g for 2 min. Remove 2 mL of the supernatant, extract the aqueous phase again with 3 mL ethyl acetate saturated with water. Combine the organic layers and evaporate them to dryness under a stream of air, reconstitute with 1 mL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm Bondapak C18
Mobile phase: MeCN:30 mM pH 4.6 sodium acetate 35:65
Flow rate: 1.5
Injection volume: 100
Detector: UV 436

CHROMATOGRAM

Retention time: 6.4
Limit of detection: 40 ng/mL

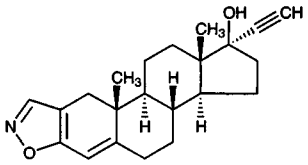
KEY WORDS

plasma; use siliconized glass; do not use plastic

REFERENCE

Schneebaum,S.; Bies,J.M.; Briele,H.A. Reversed-phase high-performance liquid chromatography of dactinomycin, *J.Chromatogr.*, **1988**, *427*, 166–171.

Danazol



Molecular formula: $C_{22}H_{27}NO_2$
Molecular weight: 337.46
CAS Registry No.: 17230-88-5
Merck Index: 2875
Lednicer No.: 2 157

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 100 µL 10 µg/mL IS + 5 mL pentane:dichloromethane 80:20, shake for 10 min on a reciprocal shaker at 150 rpm, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 µL mobile phase. Inject an aliquot onto column A and elute to waste with mobile phase, after 1.4 min direct the effluent from column A onto column B, after another 2.6 min remove column A from the circuit. Elute column B with mobile phase and monitor the effluent. Continue to elute column A with mobile phase to remove late-eluting peaks.

HPLC VARIABLES

Column: A 150 × 3.9 5 µm Spherisorb C8; B 150 × 4.6 5 µm Spherisorb ODS-2
Mobile phase: MeCN:water 55:45
Flow rate: 1.6
Detector: UV 285

CHROMATOGRAM

Retention time: 9.5
Internal standard: 1,4-androstadiene-3,17-dione (4.2)
Limit of detection: 1 ng/mL

KEY WORDS

serum; column-switching; heart-cut; pharmacokinetics

REFERENCE

Selinger,K.; Hill,H.M.; Anslow,J.A.; Gash,D. A liquid chromatographic method for the determination of danazol in human serum, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 79–84.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 0.1% solution in mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 LiChrosorb 10-RP-18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 240, 254, 284

CHROMATOGRAM

Retention time: 15.9
Limit of detection: 0.05% of danazol

OTHER SUBSTANCES

Simultaneous: impurities, ethisterone, isodanazol

REFERENCE

Balogh,G.; Csizér,.; Ferenczy,G.G.; Halmos,Z.; Herényi,B.; Horváth,P.; Laukó,A.; Görög,S. Estimation of impurity profiles of drugs and related materials. 12. Isolation and identification of an isomeric impurity in danazol, *Pharm.Res.*, **1995**, *12*, 295–298.

SAMPLE

Matrix: formulations

Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.

Flow rate: 1

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 14.0

OTHER SUBSTANCES

Simultaneous: (at UV 240 nm) boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate

Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS

tablets

REFERENCE

Lurie,I.S.; Sperling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb S5-ODS2 (A), 125 × 4 5 μm LiChrospher ODS-3 (B), 250 × 4.6 Partisil 10 ODS-3 (C)

Mobile phase: MeCN:water 65:35 (A), MeCN:MeOH:water 40:30:30 (B and C)

Flow rate: 1 (A), 1.5 (B), 2 (C)

Injection volume: 20 (A), 100 (B and C)

Detector: UV 270 (A), UV 280 (C)

CHROMATOGRAM

Internal standard: testosterone propionate

REFERENCE

Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: methyltestosterone, nandrolone, methandrostenolone, boldenone, testosterone, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 26.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: biphenyl

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeCN:MeOH:water 40:30:30
Flow rate: 1
Detector: UV 280

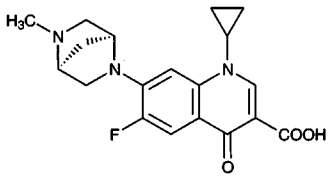
CHROMATOGRAM

Retention time: 8

REFERENCE

Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

Danofloxacin



Molecular formula: $C_{19}H_{20}FN_3O_3$, $C_{19}H_{20}FN_3O_3.CH_4O_3S$ (monomethane sulfonate)

Molecular weight: 357.38

CAS Registry No.: 112398-08-0, 119478-55-6 (monomethane sulfonate)

Merck Index: 2876

SAMPLE

Matrix: blood, gastric contents; tissue

Sample preparation: Blood. Mix 1 mL plasma with IS and MeCN, centrifuge at 2800 g for 20 min, inject an aliquot. Tissue. Centrifuge 500 mg homogenized lung tissue at 1820 g, separate the sediment, reconstitute it with 500 μL water, vortex for 20 s. Mix with 5 μg/g IS in MeCN (sample:MeCN 40:60). Vortex for 20 s, centrifuge at 1820 g. Collect the supernatant and dilute with water (sample:water 30:70). Centrifuge the mixture and inject an aliquot of the supernatant. Mesenteric lymph node, brain, interdigital skin. Centrifuge 500 mg homogenized tissue at 1820 g, mix sediment with IS, vortex, mix with 1 mL 2 M pH 8.5 K_2HPO_4, vortex, homogenize (Ultraturrax) for 20 s. Add 5 mL ethyl acetate:isopropanol 70:30, mix for 10 min. Centrifuge at 1820 g, remove 3 mL portion, dry at 50° under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. Intestinal contents. Condition a Sep-Pak C18 SPE cartridge with 3 mL MeOH and 3 mL 25 mM pH 3.0 K_2HPO_4. Centrifuge 1 g gastrointestinal contents at 1820 g, add IS, vortex, mix with 2 M pH 8.5 K_2HPO_4. Add 10 mL ethyl acetate:isopropanol 70:30, mix for 10 min, centrifuge at 1820 g, remove the supernatant, dry at 50° under nitrogen. Reconstitute the residue with 1 mL 25 mM pH 3.0 K_2HPO_4, sonicate for 2 min, vortex for 20 s. Add to the SPE cartridge, wash with 3 mL 35 mM pH 3.0 K_2HPO_4, dry under vacuum, elute with 3 mL MeOH. Dry the eluate under a stream of nitrogen at 50°, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 5 μm Techsphere C18

Mobile phase: MeCN:10 mM pH 3.0 phosphate buffer

Flow rate: 1

Detector: F ex 280 em 440

CHROMATOGRAM

Internal standard: CP.71.755 (a structural analogue of danofloxacin)

Limit of detection: 10 ng/mL (plasma), 40 ng/g (tissue, all gastrointestinal fluids)

KEY WORDS

SPE; plasma; mesenteric lymph nodes; duodenum; jejunum; ileum; colon; brain; interdigital skin; sheep; pharmacokinetics

REFERENCE

McKellar,Q.A.; Gibson,I.F.; McCormack,R.Z. Pharmacokinetics and tissue disposition of danofloxacin in sheep, *Biopharm.Drug Dispos.*, **1998**, *19*, 123–129.

SAMPLE

Matrix: tissue

Sample preparation: 500 mg Sample + 5 mL extraction solvent, mix at high speed for 10 s, homogenize at high speed (Polytron homogenizer) for 30 s. Re-suspend the tissue by mixing at high speed for 10 s, incubate in a 50 ± 5° water bath for 90 ± 10 min. Centrifuge at 1200 g for 10 min, inject a 20 μL aliquot of the supernatant. (Extraction solvent was 15 mM $HClO_4$ and 15 mM phosphoric acid in MeOH:water 50:50.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Inertsil C8 (GL Sciences, Japan)
Mobile phase: MeCN:50 mM pH 3.5 phosphate buffer 12:88 (Buffer was prepared by dissolving 6.62 g monosodium phosphate in 900 mL water, pH was adjusted to 3.5 with phosphoric acid and solution was diluted to 1 L with water.)
Column temperature: 35 ± 0.5
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 440

CHROMATOGRAM

Retention time: 18.5
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin

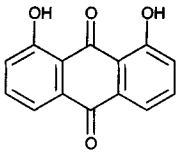
KEY WORDS

liver; muscle; kidney; fat; cow; chicken

REFERENCE

Strelevitz,T.J.; Linhares,M.C. Simultaneous determination of danofloxacin and N-desmethyldanofloxacin in cattle and chicken edible tissues by liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1996**, *675*, 243–250.

Danthron



Molecular formula: $C_{14}H_8O_4$
Molecular weight: 240.22
CAS Registry No.: 117-10-2
Merck Index: 2878

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

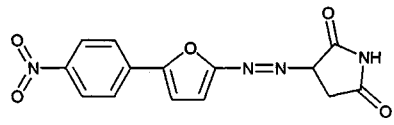
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, py-

rilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Dantrolene



Molecular formula: $C_{14}H_{10}N_4O_5$
Molecular weight: 314.26
CAS Registry No.: 7261-97-4, 24868-20-0 (sodium salt hemiheptahydrate)
Merck Index: 2879
Lednicer No.: 2 242

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL 10 μg/mL methyldantrolene in MeCN, vortex for 30 s, centrifuge at 1500 g for 5 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: MeCN:20 mM glycine 35:45, final pH adjusted to 3.6 with phosphoric acid
Flow rate: 2
Injection volume: 25
Detector: UV 375

CHROMATOGRAM

Retention time: 6.4
Internal standard: methyldantrolene (11.2) (synthesis details in paper)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: tetracycline, benzodiazepines

KEY WORDS

plasma; human; rat; pharmacokinetics

REFERENCE

Lalande,M.; Mills,P.; Peterson,R.G. Determination of dantrolene and its reduced and oxidized metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 187–191.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Blood + 1 mL MeCN, centrifuge. Remove 850 μL of the supernatant and evaporate under a stream of nitrogen, reconstitute in 25 μL 100 mM NaOH and 75 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 5 μm LiChrosorb RP8 RT, 125-4
Mobile phase: MeCN:pH 6.8 phosphate buffer 45:55
Flow rate: 1
Detector: UV 405

OTHER SUBSTANCES

Noninterfering: sodium taurocholate

KEY WORDS

rat; pharmacokinetics

REFERENCE

Poelma,F.G.J.; Tukker,J.J.; Crommelin,D.J.A. Intestinal absorption of drugs. I: The influence of taurocholate on the absorption of dantrolene in the small intestine of the rat, *J.Pharm.Sci.*, **1989**, *78*, 285–289.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.04 (A), 5.23 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

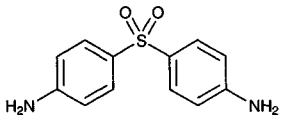
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Dapsone



Molecular formula: $C_{12}H_{12}N_2O_2S$
Molecular weight: 248.31
CAS Registry No.: 80-08-0
Merck Index: 2885
Lednicer No.: 1 139

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL Plasma with 5 μL 50 μg/mL IS in MeOH, 200 mg NaCl, 200 μL 1.5 M NaOH, and 7 mL diethyl ether. Shake for 30 min in a mechanical shaker at 220 ±10 cycles/min, centrifuge at 1000 g for 10 min, remove a 5 mL aliquot of the organic layer, evaporate under a stream of air, add 50 μL mobile phase and 50 μL n-hexane to the residue, vortex for 10 s, inject a 20 μL aliquot of the lower phase.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeOH:water 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 286

CHROMATOGRAM

Retention time: 3.82
Internal standard: phenacetin (9.1)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: monoacetyldapsone
Simultaneous: acetaminophen, aspirin, clofazimine, diazepam, thalidomide, trimethoprim, sulfamethoxazole
Noninterfering: quinine, rifampin

KEY WORDS

plasma

REFERENCE

Queiroz,R.H.; Dreossi,S.A.; Carvalho,D. A rapid, specific, and sensitive method for the determination of acetylation phenotype using dapsone, *J.Anal.Toxicol.*, **1997**, *21*, 203–207.

SAMPLE

Matrix: blood
Sample preparation: Mix 50 μL plasma, filter paper-absorbed plasma, or whole blood with 1 μg IS. Dry the filter paper-absorbed samples under air for 30 min, cut blots into small pieces. Add 200 μL ammonia to liquid or dry sample, vortex for 5 s, extract with 5 mL ethyl acetate:MTBE 50:50, centrifuge, separate, evaporate the organic phase to dryness at 37°. Reconstitute the residue with 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm CN RP-18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 17.5:81:5 containing 2 g/L l-octanesulfonic acid
Injection volume: 50

Detector: UV 274

CHROMATOGRAM
Retention time: 6
Internal standard: acetanilide (4.5)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; whole blood

REFERENCE
Mberu,E.K.; Muhia,D.K.; Minyiri,G.O.; Njonge,E.W.; Watkins,W.M. Measurement of physiological concentrations of dapsone and its monoacetyl metabolite: a miniaturised assay for liquid or filter paper-absorbed samples, *J.Chromatogr.B*, **1996**, *677*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 20 μL MeOH:60% perchloric acid 50:50, vortex for 1 min, centrifuge at 9950 g for 2 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Magnusphere C18 (Magnus Scientific)
Mobile phase: MeOH:67 mM pH 5.9 phosphate buffer 47:23
Flow rate: 1.2
Injection volume: 20
Detector: UV 295

CHROMATOGRAM
Retention time: 3.3
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Philip,P.A.; Roberts,M.S.; Rogers,H.J. A rapid method for determination of acetylation phenotype using dapsone, *Br.J.Clin.Pharmacol.*, **1984**, *17*, 465–469.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Whole blood or plasma + 25 μL monopropionyldapsone in EtOH, mix at 2200 vibrations/min for 10 min, add 100 μL 2 M NaOH, add 3 mL MTBE, shake mechanically for 15 min, centrifuge at 1200 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, vortex for 20 s, centrifuge at 1200 g for 2 min, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:25 mM pH 2.3 phosphate buffer 20:10:70
Flow rate: 1.2

Injection volume: 80
Detector: UV 286

CHROMATOGRAM
Retention time: 3.9
Internal standard: monopropionyldapsone (7.5) (Reflux dapsone with propionic anhydride in ethyl acetate for 10 min, purify by preparative TLC.)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone, pyrimethamine
Noninterfering: chloroquine, quinine, sulfamethoxazole, trimethoprim, acetaminophen
Interfering: proguanil

KEY WORDS
whole blood; plasma

REFERENCE
Lemnge,M.M.; Ronn,A.; Flachs,H.; Bygbjerg,I.C. Simultaneous determination of dapsone, monoacetyldapsone and pyrimethamine in whole blood and plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 340–346.

SAMPLE
Matrix: blood
Sample preparation: Whole blood. 150 μL Whole blood + 25 μL 800 ng/mL monopropionyldapsone + 1.2 mL 200 mM NaOH + 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot. Dried blood. Let 150 μL blood dry on filter paper. Cut paper into small pieces, add 25 μL 800 ng/mL monopropionyldapsone, add 1.2 mL 200 mM NaOH, mix gently for 30 min, add 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-ABZ (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:buffer 14:7:49 (Buffer was 25 mM phosphate adjusted to pH 2.3 with orthophosphoric acid.)
Flow rate: 1.2
Injection volume: 80
Detector: UV 286

CHROMATOGRAM
Retention time: 3.6
Internal standard: monopropionyldapsone (7.1)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone, metabolites, pyrimethamine
Noninterfering: acetaminophen, chloroquine, quinine, sulfadoxine, sulfamethoxazole, trimethoprim

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KEY WORDS
whole blood; dried blood

REFERENCE

Ronn,A.M.; Lemnge,M.M.; Angelo,H.R.; Bygbjerg,I.C. High-performance liquid chromatography determination of dapsone, monoacetyldapsone, and pyrimethamine in filter paper blood spots, *Ther.Drug Monit.*, **1995**, *17*, 79–83.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 4 μg thiopental (in MeOH) + 2 mL 34 mg/mL pH 5.5 KH_2PO_4 + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:0.68 mg/mL pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 293

CHROMATOGRAM

Retention time: 2.9
Internal standard: thiopental (6.0)

KEY WORDS

plasma

REFERENCE

Tracqui,A.; Gutbub,A.M.; Kintz,P.; Mangin,P. A case of acute dapsone poisoning: Toxicological data and review of the literature, *J.Anal.Toxicol.*, **1995**, *19*, 229–235.

SAMPLE

Matrix: blood, saliva

Sample preparation: Serum. 200 μL Serum + 500 μL mobile phase, shake for 10 min, centrifuge at 2400 g for 5 min, inject 20 μL of the organic layer. Saliva. 200 μL Saliva + 200 μL 1.4 M pH 7.5 phosphate buffer + 1 mL mobile phase, shake for 10 min, centrifuge at 2400 g for 5 min, inject a 20 μL aliquot of the organic layer.

HPLC VARIABLES

Column: 100 × 3 5 μm Chromspher Si (Chrompack)
Mobile phase: 1,2-Dichloroethane:n-butyl acetate (50% water saturated) 10:90
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 380

CHROMATOGRAM

Retention time: 0.92
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: monoacetyldapsone

KEY WORDS

serum; normal phase; pharmacokinetics

REFERENCE
Pieters,F.A.J.M.; Vincken,B.J.; Zuidema,J. Dapsone and monoacetyldapsone determined in serum and saliva by a sensitive high-performance liquid chromatographic method with a single extraction step, *J.Chromatogr.*, **1987**, *422*, 322–327.

SAMPLE
Matrix: blood, saliva
Sample preparation: Serum. 200 μL Serum + 100 μL 100 μg/mL diazoxide in buffer + 50 μL 20% perchloric acid, vortex, centrifuge at 2000 g for 5 min, inject a 250 μL aliquot of the supernatant onto column A, then inject 700 μL buffer onto column A, elute the contents of column A onto column B with mobile phase for 1 min, remove column A from circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with 2 mL MeOH:buffer 50:50 then forward flush with 1 mL buffer. Saliva. Centrifuge saliva at 3000 g for 4 min. 100 μL Supernatant + 150 μL water + 100 μL 5 μg/mL diazoxide in buffer, vortex, inject a 250 μL aliquot onto column A, then inject 700 μL buffer onto column A, elute the contents of column A onto column B with mobile phase for 1 min, remove column A from circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with 2 mL MeOH:buffer 50:50 then forward flush with 1 mL buffer. (Buffer was 50 mM pH 4.6 $(NH_4)H_2PO_4$.)

HPLC VARIABLES
Column: A 30 mm long 30-40 μm C18; B 10 × 4.6 5 μm Spherisorb S5 ODS-1 C18 end-capped + 250 × 4.6 5 μm Spherisorb S5 ODS-1 C18 end-capped
Mobile phase: MeCN:buffer 12:88 (Buffer was 50 mM pH 4.6 $(NH_4)H_2PO_4$.)
Column temperature: 40
Flow rate: 2
Injection volume: 250
Detector: UV 295

CHROMATOGRAM
Retention time: 6.8
Internal standard: diazoxide (8.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; column-switching

REFERENCE
Moncrieff,J. Determination of dapsone in serum and saliva using reversed-phase high-performance liquid chromatography with ultraviolet or electrochemical detection, *J.Chromatogr.B*, **1994**, *654*, 103–110.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 100-500 μL urine + 50 μL 20 μg/mL m-aminophenyl sulfone in MeOH + 100 μL 1 M NaOH + 350 μL water + 3 mL dichloromethane, vortex for 1 min, centrifuge at 950 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 30-50 μL mobile phase, inject a 10-15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:water:acetic acid 25:73:2
Column temperature: 40

Flow rate: 1.3
Injection volume: 10-15
Detector: UV 250

CHROMATOGRAM
Retention time: 4.5
Internal standard: m-aminophenyl sulfone (6.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone

KEY WORDS
plasma

REFERENCE
Horai,Y.; Ishizaki,T. Rapid and sensitive liquid chromatographic method for the determination of dapsone and monoacetyldapsone in plasma and urine, *J.Chromatogr.*, **1985**, *345*, 447–452.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.583

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 11.0

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.81

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, primaquine, pyrimethamine, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole

REFERENCE
Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1317–1323.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

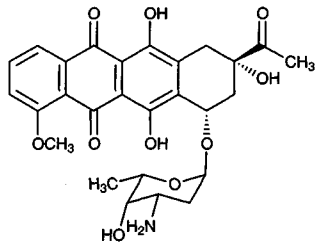
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, na-

lorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Daunorubicin



Molecular formula: $C_{27}H_{29}NO_{10}$
Molecular weight: 527.53
CAS Registry No.: 20830-81-3, 23541-50-6 (HCl)
Merck Index: 2890

SAMPLE

Matrix: blood
Sample preparation: Plasma + 1 mL pH 8.4 phosphate buffer + 8 mL chloroform:1-heptanol 90:10, shake mechanically for 30 min, centrifuge at 3300 rpm for 10 min. Remove the lower organic layer and evaporate it to 2 mL under a stream of nitrogen. Add the residue to 200 µL 300 mM phosphoric acid, vortex. Remove the aqueous phase and add it to 2 mL n-hexane, vortex, centrifuge, inject a 100-150 µL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 7 µm Lichrosorb RP-18
Mobile phase: MeOH:water 70:30 containing 0.5% acetic acid and 2.5 mM sodium heptanesulfonate
Flow rate: 1.2
Injection volume: 100-150
Detector: UV 254

CHROMATOGRAM

Internal standard: daunorubicin

OTHER SUBSTANCES

Extracted: epirubicin

KEY WORDS

plasma; daunorubicin is IS

REFERENCE

Hu,O.Y.-P.; Chang,S.-P.; Jame,J.-M.; Chen,K.-Y. Pharmacokinetic and pharmacodynamic studies with 4'-epi-doxorubicin in nasopharyngeal carcinoma patients, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 332–337.

SAMPLE

Matrix: blood
Sample preparation: Condition a C2 SPE cartridge with 1 mL MeOH, 500 µL water and 500 µL buffer. 1 mL Plasma + 500 µL water, mix, add to SPE cartridge, wash with 500 µL buffer, elute contents of SPE cartridge with mobile phase onto column for 1 min, remove SPE cartridge, elute column with mobile phase, monitor the effluent. (Buffer was 19 mM NaH_2PO_4 adjusted to pH 4.0 with 100 mM phosphoric acid:MeCN 90:10.)

HPLC VARIABLES

Guard column: 50 × 5 10 µm LiChrosorb RP-18
Column: 100 × 5 5 µm Apex II ODS (Jones Chromatography)
Mobile phase: MeCN:buffer 1:2.25 (Buffer was 19 mM NaH_2PO_4 adjusted to pH 4.0 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 1000
Detector: F ex 480 em 580

CHROMATOGRAM
Retention time: 22
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: epirubicin

KEY WORDS
plasma; SPE; daunorubicin is IS

REFERENCE
Dobbs,N.A.; Twelves,C.J. Measurement of epidoxorubicin and its metabolites by high-performance liquid chromatography using an advanced automated sample processor, *J.Chromatogr.*, **1991**, *572*, 211–217.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or blood + 3 mL 100 mM pH 9.5 ammonia-ammonium chloride buffer + 13.5 mL chloroform:MeOH 2:1, shake mechanically for 30 min, centrifuge at 3000 g for 10 min, repeat the extraction with 9 mL chloroform. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 3 mL chloroform:MeOH 2:1, evaporate this mixture, reconstitute the residue in 300 μL mobile phase, centrifuge a 75 μL aliquot at 10000 g for 1 min, inject the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm STR ODS-M (Shimadzu)
Mobile phase: MeCN:buffer 30:70 (Buffer was 200 mM acetic acid-ammonium formate, pH 4.0.)
Column temperature: 22
Flow rate: 0.7
Injection volume: 75
Detector: F ex 470 em 550

CHROMATOGRAM
Retention time: 16.2
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: pirarubicin, doxorubicin

KEY WORDS
plasma; whole blood; daunorubicin is IS

REFERENCE
Nagasawa,K.; Yokoyama,T.; Ohnishi,N.; Iwakawa,S.; Okumura,K.; Kosaka,Y.; Sano,K.; Murakami,R.; Nakamura,H. Pharmacokinetics of pirarubicin in pediatric patients, *J.Pharmacobiodyn.*, **1991**, *14*, 222–230.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL Bondelut C18 SPE cartridge with 3 mL MeOH and 3 ml MeOH:phosphate buffer 1:2. 1 mL Plasma + 1 mL 10 mM pH 8 phosphate buffer containing 600 nM tetrabutylammonium bromide + 1 mL MeOH, add to the SPE cartridge, wash with 4 mL MeOH:water 25:75, elute with 3 mL 30 mM phosphoric acid in MeOH. Add the eluate to 100 μL 100 mM KH_2PO_4, evaporate to 100-400 μL under vacuum at 25°, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: Gradient. A was MeCN:10 mM KH_2PO_4 22:78. B was MeCN:10 mM KH_2PO_4 containing 6 mM phosphoric acid 70:30. A:B from 90:10 to 80:20 over 9 min.
Injection volume: 10-100
Detector: F ex 470 em 580

CHROMATOGRAM
Retention time: 10.1
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: idarubicin

KEY WORDS
plasma; SPE; daunorubicin is IS

REFERENCE
Camaggi,C.M.; Carisi,P.; Strocchi,E.; Pannuti,F. High-performance liquid chromatographic analysis of idarubicin and fluorescent metabolites in biological fluids, *Cancer Chemother.Pharmacol.*, **1992**, *30*, 303–306.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL mobile phase, extract with 3 mL MeCN for 10 min, add 100 mg NaCl, shake for 5 min, centrifuge at 995 g for 15 min, let stand at -20° for 1 h. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Spherisorb phenyl
Column: 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:30 mM citrate buffer adjusted to pH 4 with formic acid 30:70
Column temperature: 50
Flow rate: 1.5
Injection volume: 100
Detector: F ex 480 em 590

CHROMATOGRAM
Retention time: 8.5
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: pirarubicin, doxorubicin, doxorubicinol

KEY WORDS
plasma; daunorubicin is IS

REFERENCE
Jacquet,J.M.; Galtier,M.; Bressolle,F.; Jourdan,J. A sensitive and reproducible HPLC assay for doxorubicin and pirarubicin, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 343–348.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum

at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 234

CHROMATOGRAM

Retention time: 8.90
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, bile, feces, tissue, urine
Sample preparation: Homogenize tissue in 4% BSA in water to a final concentration of 0.05-2 g/mL. Homogenize feces in 4% BSA in water to a final concentration of 0.03-1 g/mL. Dilute feces homogenate 20-fold, urine 100-fold, and bile 20-fold with blank human plasma. 200 μL Sample + 200 μL 6% (w/v) pH 9.5 borate buffer + 100 μL pH 2.05 water, vortex. Mix with 1 mL chloroform:1-propanol 20:80 for 5 min. (Caution! Chloroform is a carcinogen!) Centrifuge at 3000 g at 4° for 10 min. Remove the organic layer and evaporate it under reduced pressure at 43°. Reconstitute the residue in 100 μL MeCN:THF 40:1, vortex for 20 s, sonicate for 5 min. Add 300 μL water acidified to pH 2.05, vortex, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 pellicular RP material
Column: 100 × 3 7μm Lichrosorb RP-8
Mobile phase: MeCN:THF:water adjusted to pH 2.05 with perchloric acid 30:1:80
Flow rate: 0.4
Injection volume: 50
Detector: F ex 460 em 550

CHROMATOGRAM
Retention time: 12
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
mouse; plasma; brain; muscle; colon; cecum; small intestine; stomach; liver; gall bladder; kidney; lung; spleen; heart; ovary; uterus; breast; testis; epididymis; eye; daunorubicin is IS

REFERENCE
van Asperen,J.; van Tellingen,O.; Beijnen,J.H. Determination of doxorubicin and metabolites in murine specimens by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *712*, 129–143.

SAMPLE
Matrix: blood, cells
Sample preparation: Thaw cell samples, sonicate (Branson B-12) at 50 W for 20 s. 400 μL Cell sample or plasma + 200 μL 100 mM pH 9.3 borate buffer, add 1.8 mL chloroform:MeOH 80:20, extract, inject a 200-500 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 250 × 4 Lichrosorb Si-60
Mobile phase: Chloroform:MeOH:glacial acetic acid:0.3 mM magnesium chloride 72:21:2:3
Flow rate: 1.5
Injection volume: 200-500
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 3.3
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: doxorubicin, epirubicin

KEY WORDS
plasma; normal phase; daunorubicin is IS

REFERENCE
Tidefelt,U.; Sundman-Engberg,B.; Paul,C. Comparison of the intracellular pharmacokinetics of doxorubicin and 4'-epi-doxorubicin in patients with acute leukemia, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 225–229.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. Serum + 8 mL chloroform:isopropanol 50:50, extract, centrifuge at 3000 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot. Tissue. Homogenize tissue in water, add 30 μL silver nitrate (33%), extract with 8 mL isopropanol. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 300 mm long 10 μm μBondapak C18
Mobile phase: MeCN:water:100 mM phosphoric acid 37:37:26
Flow rate: 1.5
Injection volume: 5-20
Detector: F ex 475 em 580

CHROMATOGRAM
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
serum; rat; daunorubicin is IS; heart; liver; kidney; adrenal; brain; intestine; mouse

REFERENCE
Colombo,T.; Zucchetti,M.; D'Incalci,M. Cyclosporin A markedly changes the distribution of doxorubicin in mice and rats, *J.Pharmacol.Exp.Ther.*, **1994**, *269*, 22–27.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 150 μL Plasma + 150 μL MeCN, vortex for 10 s. Centrifuge at 3000 rpm for 5 min. Remove 200 μL of the organic layer, evaporate under reduced pressure, reconstitute the residue in 100 μL 100 mM pH 3 monobasic phosphate buffer. Inject a 10 μL aliquot. Urine. Directly inject a 10 μL aliquot. (Silanize glassware with 3% dichlorodimethylsilane in toluene, rinse with MeOH before use.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS/TM silica (Tosoh Co., Japan)
Mobile phase: MeCN:buffer 35:65 (Buffer was 100 mM monobasic phosphate (sic) containing 0.3% heptafluorobutyric acid, adjusted to pH 3 with NaOH. At the end of the analysis, wash the column with MeOH and MeOH:water 50:50.)
Flow rate: 1
Injection volume: 10
Detector: F ex 460 em 555

CHROMATOGRAM
Retention time: 9.8
Limit of detection: 28 nM
Limit of quantitation: 2.5 μM

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
plasma

REFERENCE
Emara,S.; Morita,I.; Tamura,K.; Razee,S.; Masujima,T.; Mohamed,H.A.; El Gizawy,S.M.; El Rabbat,N.A. Utility of ion-pair chromatography for analysis of some anthracyclines in plasma and urine, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 681–692.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 3 mL MeOH, 3 mL MeOH:water 50:50, and 10 mL 50 mM pH 8.9 Na_2HPO_4. 1 mL Plasma or urine + 2 mL 0.9% NaCl, mix, add to the SPE cartridge, wash with 3 mL 50 mM pH 8.9 Na_2HPO_4, elute with four 500 μL aliquots of chloroform:MeOH 2:1. Evaporate the eluates to dryness under vacuum while centrifuging, dissolve the residue in 200 μL MeCN:7 mM pH 2.6 Na_2HPO_4 40:60, vortex gently, centrifuge at 3000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 μBondapak phenyl Radial-Pak
Mobile phase: MeCN:buffer 30:70 (Buffer was 7 mM Na_2HPO_4 adjusted to pH 2.6 with formic acid.)
Flow rate: 3
Detector: F ex 480 em 550

CHROMATOGRAM
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: epirubicin

KEY WORDS
plasma; SPE; daunorubicin is IS

REFERENCE
Tjuljandin,S.A.; Doig,R.G.; Sobol,M.M.; Watson,D.M.; Sheridan,W.P.; Morstyn,G.; Mihaly,G.; Green,M.D. Pharmacokinetics and toxicity of two schedules of high dose epirubicin, *Cancer Res.*, **1990**, *50*, 5095–5101.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + daunorubicinone + 5 mL chloroform:isopropanol 75:25, shake mechanically for 30 min, centrifuge at 4° at 1200 g for 15 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at room temperature. Add the aqueous phase to 2 mL 50 (plasma) or 500 (urine) mM pH 8.4 borate buffer, add 5 mL chloroform:isopropanol 75:25, shake mechanically for 30 min, centrifuge at 4° at 1200 g for 15 min. Remove the lower organic layer and add it to the residue from the first extraction, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 600 μL MeOH:500 mM phosphoric acid 50:50, vortex for 30 s,

add 2 mL N-hexane, vortex for 30 s, centrifuge, inject an aliquot of the aqueous phase (omit the hexane wash for urine samples).

HPLC VARIABLES
Guard column: 30-38 μm pellicular ODS (Whatman)
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:10 mM pH 1.4 phosphate buffer 25:10:65
Flow rate: 0.58
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 25
Internal standard: daunorubicin (25), daunorubicinone (35)

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
plasma; daunorubicin is IS

REFERENCE
Fraier,D.; Frigerio,E.; Pianezzola,E.; Strolin Benedetti,M.; Cassidy,J.; Vasey,P. A sensitive procedure for the quantitation of free and N-(2-hydroxypropyl)methacrylamide polymer-bound doxorubicin (PK1) and some of its metabolites, 13-dihydrodoxorubicin, 13-dihydrooxorubicinone and doxorubicinone, in human plasma and urine by reversed-phase HPLC with fluorimetric detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 625–633.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2 × 10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

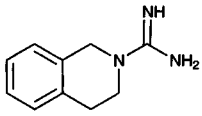
CHROMATOGRAM
Retention time: 4.0
Internal standard: daunorubicin
Limit of detection: 2 pmol
Limit of quantitation: 7 pmol

OTHER SUBSTANCES
Extracted: altretamine, doxorubicin, verapamil, S 9788

KEY WORDS
human; cells; epidermoid carcinoma; daunorubicin is IS

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

Debrisoquin



Molecular formula: $C_{10}H_{13}N_3$
Molecular weight: 175.23
CAS Registry No.: 1131-64-2, 581-88-4 (sulfate)
Merck Index: 2901
Lednicer No.: 2 374

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 262

CHROMATOGRAM

Retention time: 4.07
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Plasma. 1 mL Plasma + IS + 1 mL MeOH + 1 mL 2 M HCl, mix, centrifuge. Remove the supernatant and add it to 1 mL 2 M HCl, cool, wash with 8 mL diethyl ether. Add the aqueous layer to 1 mL 5 M NaOH. Remove a 1 mL aliquot and add it to 500 μL saturated sodium bicarbonate solution, add 500 μL MeOH, add 500 μL acetylacetone, heat at 96° for 2.5 h, cool to room temperature, add 3 mL 5 M NaOH, extract with 8 mL diethyl ether. Remove the organic layer and add it to 500 μL 2 M HCl, shake mechanically for 15 min, centrifuge. Remove the aqueous layer and add it to 500 μL 5 M NaOH, cool, extract with 8 mL diethyl ether. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 20 μL MeOH, inject an aliquot. Saliva, urine. 1 mL Saliva or urine + 2 (saliva) or 10 (urine) μg IS in MeOH + 500 μL saturated sodium bicarbonate solution + 500 μL MeOH + 500 μL acetylacetone, heat at 96° for 2.5 h, cool to room temperature, add 3 mL 5 M NaOH, extract with 8 mL diethyl ether. Remove the organic layer and add it to 500 μL 2 M HCl, shake mechanically for 15 min, centrifuge. Remove the aqueous layer and add it to 500 μL 5 M NaOH, cool, extract with 8 mL diethyl ether. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 20 μL MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: C8 (Merck)

Column: 100 × 8 μBondapak C18 in a Z-module

Mobile phase: MeOH:water containing 10 mM sodium 1-pentanesulfonate, adjusted to pH 3.5 with orthophosphoric acid

Flow rate: 1.5

Injection volume: 25-200

Detector: UV 248

CHROMATOGRAM

Retention time: 7.4

Internal standard: guanoxan hemisulfate (Pfizer) (5.6)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

derivatization; silanize all glassware with hexamethyldisilazane; plasma; comparison with GC; pharmacokinetics

REFERENCE

Chan,K. Comparison of gas chromatographic and high-performance liquid chromatographic assays for the determination of debrisoquine and its 4-hydroxy metabolite in human fluids, *J.Chromatogr.*, **1988**, *425*, 311–321.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide,

sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Condition a C18 SPE cartridge with 4 mL MeOH and 4 mL water. Filter (0.45 μm) urine, adjust pH of filtrate to 5 with 100 mM HCl, add a 1 mL aliquot to the SPE cartridge, wash with 3 mL water, wash with 1 mL MeOH:water 10:90, elute with 1 mL MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 Spherisorb C8

Column: 250 × 4.6 5 μm Spherisorb C8

Mobile phase: MeCN:buffer 70:30 (Buffer was 8 mM KH_2PO_4 adjusted to pH 5 with 2 M KOH.)

Flow rate: 1.5

Detector: UV 208

CHROMATOGRAM

Retention time: 7.5

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, 4-hydroxydebrisoquine

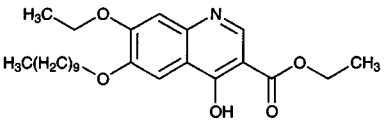
KEY WORDS

comparison with capillary electrophoresis; SPE

REFERENCE

Cifuentes,A.; Valencia,J.; Sanz,E.; Sánchez,M.J.; Rodríguez-Delgado,M.A. Separation and quantitation of debrisoquine and 4-hydroxydebrisoquine in human urine by capillary electrophoresis and high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 389–396.

Decoquinate



Molecular formula: $C_{24}H_{35}NO_5$
Molecular weight: 417.55
CAS Registry No.: 18507-89-6
Merck Index: 2910
Lednicer No.: 2 368

SAMPLE

Matrix: feed
Sample preparation: 10 g Feed + 50 mL calcium chloride in MeOH, shake vigorously for 20 min, centrifuge at 1000 rpm for 2 min, remove the supernatant and extract the residue twice with 50 mL portions of calcium chloride in MeOH. Combine all the supernatants and add them to 100 mL 500 mM HCl, extract with three 20 mL aliquots of chloroform. Combine the chloroform extracts and wash them with 20 mL water. Extract the aqueous layer three times with 15 mL portions of chloroform. Combine all the chloroform layers and evaporate them to dryness under vacuum at 40°. Reconstitute the residue in MeOH: chloroform 50:50, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb ODS
Mobile phase: Gradient. A was MeOH:water 95:5 containing 0.001% acetic acid and 1% magnesium sulfate heptahydrate. B was MeOH. A:B 5:95 to 80:20 over 10 min (convex gradient, code 0.3, Perkin-Elmer) then to 95:5 over 5 min (linear), then to 5:95 over 5 min (linear), re-equilibrate for 10 min.
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: F ex 314 em 390

CHROMATOGRAM

Retention time: 9
Limit of quantitation: <1000 ng/mL

OTHER SUBSTANCES

Simultaneous: nequinate, buquinolate
Noninterfering: amprolium, arprinocid, clopidol, dimetridazole, sulfaquinoxaline

REFERENCE

Hobson-Frohock,A. Determination of decoquinate in poultry feed, *Analyst*, **1982**, *107*, 1195–1199.

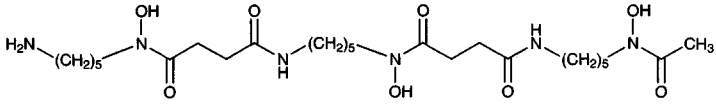
Deferoxamine

Molecular formula: $C_{25}H_{48}N_6O_8$

Molecular weight: 560.69

CAS Registry No.: 70-51-9, 1950-39-6 (HCl), 138-14-7 (mesylate)

Merck Index: 2914



SAMPLE

Matrix: blood, dialysate, urine

Sample preparation: Plasma. Condition a 3 mL C18 SPE cartridge (Varian) with 3 mL MeOH and 3 mL 50 mM pH 9.0 Tris buffer. Mix 1 mL 100 mM pH 9.0 Tris buffer with 1 mL plasma, add to the SPE cartridge. Wash with 1 mL 50 mM pH 9.0 Tris buffer, apply a vacuum of 100 mbar for 10 min and elute with two 500 μL portions of MeOH:acetic acid:water 90:5:5. Evaporate the eluate to dryness under a stream of nitrogen and reconstitute the dry residue in 1 mL 50 mM pH 9.0 Tris buffer, vortex, centrifuge at 3000 g for 10 min. Inject a 10 μL aliquot of the supernatant. Dialysate. Condition a 3 mL C18 SPE cartridge (Varian) with 3 mL MeOH and 3 mL 50 mM pH 9.0 Tris buffer. Adjust 100 mL dialysate to pH 9.0 with 1 M pH 9.0 Tris buffer, add to the SPE cartridge. Wash with 1 mL 50 mM pH 9.0 Tris buffer, apply a vacuum of 100 mbar for 10 min and elute with two 500 μL portions of MeOH:acetic acid:water 90:5:5. Evaporate the eluate to dryness under a stream of nitrogen and reconstitute the dry residue in 1 mL 50 mM pH 9.0 Tris buffer, vortex, centrifuge at 3000 g for 10 min. Inject a 10 μL aliquot of the supernatant. Urine. Dilute urine 9 fold with 50 mM pH 9.0 Tris buffer and inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm ODS-silica (Shandon) + 250 × 4.6 5 μm ODS-silica (Shandon)

Mobile phase: MeCN:DMF:water 185:40:1000 (mL?) containing 600 mg sodium heptanesulfonate, 400 mg ammonium sulfate, and 100 mg disodium EDTA, adjusted to pH 1.5 with 25% sulfuric acid

Flow rate: 1

Injection volume: 10

Detector: UV 430 following post-column reaction. The column effluent mixed with mobile phase:reagent 25:3 pumped at 1 mL/min and the mixture flowed through a 20 cm capillary to the detector. (Prepare the reagent as follows. Dissolve 40 g ferric nitrate nonahydrate and 12.6 g sodium fluoride in 1000 mL water containing 2 mL concentrated nitric acid.)

CHROMATOGRAM

Retention time: 13.49

Limit of detection: 313 ng/mL

Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES

Extracted: metabolites, feroxamine, aluminoxamine

KEY WORDS

plasma; SPE; post-column reaction

REFERENCE

Kraemer,H.-J.; Breithaupt,H. Quantification of desferrioxamine, ferrioxamine and aluminoxamine by post-column derivatization high-performance liquid chromatography. Non-linear calibration resulting from second-order reaction kinetics, *J.Chromatogr.B*, **1998**, *710*, 191–204.

SAMPLE

Matrix: blood, tissue

Sample preparation: Condition a Sep-Pak Plus SPE cartridge with 3 mL MeOH:acetic acid 80:20, 3 mL MeOH, and 9 mL water. Homogenize (motorized Potter-Elvehjem) rat lung with 10 mL buffer. 500 μL Plasma or lung homogenate + 10 μL 250 mM $FeCl_3$ solution, store at -85° for 2 days then in liquid nitrogen until analysis, thaw, add 25 μL 60 μg/mL IS, add 500 μL chloroform, centrifuge at 2000 g for 5 min, add a 400 μL aliquot of the upper aqueous layer to the SPE cartridge, wash with 5 mL water, wash with 500 μL MeOH, elute with 1.5 mL MeOH:acetic acid 80:20. Evaporate the eluate to dryness under reduced pressure, reconstitute with 200 μL 50 mM pH 8.0 NaH_2PO_4, sonicate, centrifuge at 2000 g for 5 min, inject a 100 μL aliquot. (Buffer was 25.6 mM Na_2HPO_4 containing 3.72 mM NaH_2PO_4, 0.74 mM KH_2PO_4, 31 mM NaCl, 1.34 mM KCl, 0.025 mM $CaCl_2$, 0.025 mM $MgCl_2$, and 50 mM dithiothreitol, pH 7.4.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C8

Mobile phase: Gradient. MeOH:water:50 mM pH 8.0 NaH_2PO_4 from 0:0:100 to 25:25:50 over 18 min, to 40:60:0 over 4 min, return to initial conditions over 3 min.

Flow rate: 1.2 for 18 min then 1

Injection volume: 100

Detector: UV 427.5

CHROMATOGRAM

Retention time: 12 (as Fe complex, feroxamine)

Internal standard: adrenochrome semicarbazone sulfonate (14)

Limit of quantitation: 330 ng/mL

KEY WORDS

rat; plasma; lung; SPE; derivatization; complexation

REFERENCE

Merali,S.; Chin,K.; Del Angel,L.; Grady,R.W.; Armstrong,M.; Clarkson,A.B.,Jr. Clinically achievable plasma deferoxamine concentrations are therapeutic in a rat model of pneumocystis carinii pneumonia, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2023–2026.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH: acetic acid 80:20, 5 mL MeOH, and 10 mL water. 600 μL Plasma +12 μL 50 mM ferric chloride in water + 600 μL chloroform, vortex for 1 min, centrifuge at 8000 g for 10 min. Remove 500 μL of the upper aqueous layer and add it to the SPE cartridge, wash with three 5 mL portions of water, wash with 400 μL MeOH, elute with 1.5 mL MeOH:acetic acid 80:20. Evaporate the eluate under vacuum, reconstitute in 100 μL mobile phase: acetic acid 80:20, vortex for 30 s, sonicate for 30 s, centrifuge at 8000 g for 2 min, inject an aliquot of the supernatant. Alternatively, 3 mL Plasma + 50 μL 50 mM ferric chloride, saturate with NaCl, vortex for 30 s, add 600 μL benzyl alcohol, vortex twice for 30 s, centrifuge at 2000 g for 15 min, break up layers with glass rod, centrifuge at 2000 g for 15 min, inject an aliquot of the benzyl alcohol layer. Urine. 5 mL Urine + 100 μL 50 mM ferric chloride, saturate with NaCl, extract with 1 mL benzyl alcohol, inject an aliquot of the benzyl alcohol layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve spherical porous silica (Waters)

Mobile phase: MeCN:MeOH:n-butanol:water:5 M sodium perchlorate:perchloric acid 500: 300:100:100:1.5:0.05

Injection volume: 20

Detector: UV 435

CHROMATOGRAM
Retention time: 8.70 mL (elution volume as Fe complex)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
use only PTFE and titanium tubing and fittings; pharmacokinetics; SPE; human; pig; derivatization; plasma; complexation

REFERENCE
Kruck,T.P.A.; Teichert-Kuliszewska,K.; Fisher,E.; Kalow,W.; McLachlan,D.R. High-performance liquid chromatographic analysis of desferrioxamine. Pharmacokinetic and metabolic studies, *J.Chromatogr.*, **1988**, *433*, 207–216.

SAMPLE
Matrix: blood, urine
Sample preparation: Filter (Amicon Centriflow CF-25) serum. Inject a 30 μL aliquot of serum ultrafiltrate or urine.

HPLC VARIABLES
Guard column: 10 μm Bondapak C18
Column: 100 × 8 4 μm Novapak C18
Mobile phase: Gradient. A was 20 mM pH 7.0 phosphate buffer containing 2 mM nitrilotriacetic acid. B was MeCN:water containing 20 mM phosphate and 2 mM nitrilotriacetic acid, pH 7.0. A:B 100:0 for 1 min, to 82:18 over 19 min, to 75:25 over 4 min, to 65:35 over 4 min, maintain at 65:35 for 3 min, return to 100:0 over 1 min, re-equilibrate for 10 min.
Flow rate: 1.5
Injection volume: 30
Detector: UV 235

CHROMATOGRAM
Retention time: 29
Limit of detection: 1500 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, ferrioxamine

KEY WORDS
serum

REFERENCE
Singh,S.; Hider,R.C.; Porter,J.B. Separation and identification of desferrioxamine and its iron chelating metabolites by high-performance liquid chromatography and fast atom bombardment mass spectrometry: Choice of complexing agent and application to biological fluids, *Anal.Biochem.*, **1990**, *187*, 212–219.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 250 mg/mL solution in sterile water for injection, dilute 1:1000 with mobile phase and inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 500 mM sodium phosphate, adjusted to pH 6.6 containing 4 mM EDTA and 1 M ammonium acetate.)
Flow rate: 1

Detector: UV 240

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution of deferoxamine in water, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 3 5 μm Adsorbosphere C18 glass column
Mobile phase: MeCN:MeOH:butanol:8 mM pH 7.5 phosphate buffer 50:30:10:10
Flow rate: 1
Injection volume: 15 (PTFE sample loop)
Detector: UV 215

CHROMATOGRAM
Retention time: 6.4
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: ferrioxamine

KEY WORDS
use metal-free fittings; water

REFERENCE
Venkataram,S.; Rahman,Y.E. High-performance liquid chromatographic analysis of desferrioxamine using a metal-free system, *J.Chromatogr.*, **1987**, *411*, 494–497.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize (Potter-Elvehjem) tissue with three volumes 100 mM pH 7.4 Tris-HCl buffer, centrifuge at 10000 g for 15 min, add 1 mL supernatant to SPE cartridge, wash with 1 mL water, elute with 1.8 mL MeOH. Filter (0.45 μm) eluate, evaporate under vacuum at 50°, reconstitute in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Spherisorb C18
Mobile phase: MeCN:20 mM pH 6.6 sodium phosphate buffer 10:90 containing 4 mM EDTA and 1 mM ammonium acetate
Flow rate: 1
Injection volume: 20

Detector: UV 226

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: ferrioxamine (UV 430)

KEY WORDS
rabbit; liver; lung; spleen; heart; brain; kidney; SPE

REFERENCE
Gower,J.D.; Healing,G.; Green,C.J. Determination of desferrioxamine-available iron in biological tissues by high-pressure liquid chromatography, *Anal.Biochem.*, **1989**, *180*, 126–130.

Defibrotide

Molecular weight: 45000-50000

CAS Registry No.: 83712-60-1

Merck Index: 2915

SAMPLE

Matrix: blood

Sample preparation: Heat at 70° for a few min, cool to 37°, add 12 mg trypsin, heat at 37° for 5.5 h, dilute with 10 mM pH 7.0 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: Shodex Ionpak, Model S-400S (Showa Denko; Macherey-Nagel)

Mobile phase: 10 mM pH 7.0 phosphate buffer

Detector: UV 260

CHROMATOGRAM

Internal standard: fructose (UV 195)

KEY WORDS

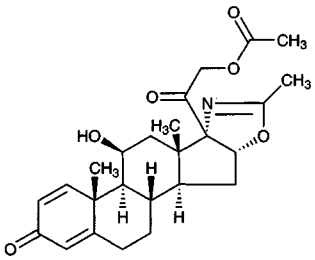
plasma; rabbit; pharmacokinetics

REFERENCE

Porta,R.; Moltrasio,D.; Pescador,R.; Lanzarotti,E.; Mantovani,M.; Prino,G. High-performance liquid chromatography determination of polydeoxyribonucleotides in plasma: its application to the determination of defibrotide's pharmacokinetics in the rabbit, *Anal.Biochem.*, **1992**, *204*, 143–146.

Deflazacort

Molecular formula: $C_{25}H_{31}NO_6$
Molecular weight: 441.52
CAS Registry No.: 14484-47-0
Merck Index: 2916



SAMPLE

Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 µL 5 µg/mL IS in MeOH, inject 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil 5-ODS
Mobile phase: THF:water 23:77
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 27
Internal standard: methylprednisolone (22)
Limit of detection: 0.12 ng

OTHER SUBSTANCES

Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deoxycorticosterone, dexamethasone, fludrocortisone, fludrocortisone acetate, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide, triamcinolone

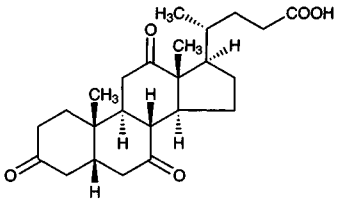
KEY WORDS

SPE also discussed

REFERENCE

Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Extraction and high-performance liquid chromatographic separation of deflazacort and its metabolite 21-hydroxydeflazacort. Application to urine samples, *J.Chromatogr.B*, **1994**, *657*, 248–253.

Dehydrocholic acid



Molecular formula: $C_{24}H_{34}O_5$
Molecular weight: 402.53
CAS Registry No.: 81-23-2, 145-41-5 (sodium salt)
Merck Index: 2922

SAMPLE

Matrix: solutions

Sample preparation: Mix sample with 400 µL 5 mM DBD-PZ in MeCn containing 70 mM diethylphosphorocyanidate, react for 6 h, inject a 1 µL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES

Column: 150 × 4.6 5 µm Inertsil ODS-2

Mobile phase: MeCN:water 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 10
Limit of detection: 13 fmol

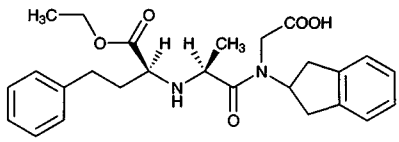
OTHER SUBSTANCES

Simultaneous: ursodiol

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

Delapril



Molecular formula: $C_{26}H_{32}N_2O_5$
Molecular weight: 452.55
CAS Registry No.: 83435-66-9, 83435-67-0 (HCl)
Merck Index: 2928
Lednicer No.: 4 58

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 1 mL Serum + 500 μL water + 3 mL MeCN, mix, centrifuge at 3000 rpm for 5 min, concentrate supernatant to about 1 mL under a stream of nitrogen. Add this concentrate to 1 mL 1% sodium bicarbonate, wash aqueous layer with 3 mL ether, add aqueous layer to 120 μL 1 M HCl and 1 mL saturated KH_2PO_4, wash aqueous layer with 5 mL hexane, extract aqueous layer with 5 mL ether, centrifuge at 3000 rpm for 5 min. Add the organic layer to 1.5 mL 1% sodium bicarbonate, shake, centrifuge at 3000 rpm for 5 min, discard the organic layer, evaporate residual ether with a stream of nitrogen, add 2 mL saturated KH_2HPO_4, add to Sep Pak C18 SPE cartridge, wash with 10 mL MeOH:water 15:85, elute with 4 mL MeOH:water 75:25, evaporate eluate to dryness under a stream of nitrogen, reconstitute in 200 μL mobile phase, inject an aliquot. Urine. 1 mL Urine + 1 mL 1% sodium bicarbonate, wash with 3 mL ether, centrifuge at 3000 rpm for 5 min, add 120 μL 1 M HCl and 1 mL saturated KH_2PO_4, wash with 5 mL hexane, extract with 5 mL ether, centrifuge at 3000 rpm for 5 min. Add the organic layer to 1.5 mL 1% sodium bicarbonate, shake, centrifuge at 3000 rpm for 5 min. Remove the aqueous layer and evaporate residual ether with a stream of nitrogen, add 2 mL saturated KH_2HPO_4, add to Sep Pak C18 SPE cartridge, wash with 10 mL MeOH:water 15:85, elute with 4 mL MeOH:water 75:25, evaporate eluate to dryness under a stream of nitrogen, reconstitute in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 10 × 4 10 μm LS-410 octadecyl-bonded silica (Toyo Soda)
Column: 300 × 4 10 μm LS-410 octadecyl-bonded silica (Toyo Soda)
Mobile phase: MeCN:100 mM KH_2PO_4 45:55
Flow rate: 0.7
Injection volume: 10-20
Detector: UV 210

CHROMATOGRAM

Retention time: 16
Limit of detection: 50 ng/mL (urine), 10 ng/mL (serum)

OTHER SUBSTANCES

Extracted: metabolites

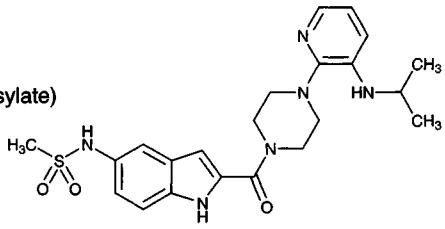
KEY WORDS

serum; SPE; pharmacokinetics

REFERENCE

Ito,H.; Yasumatsu,M.; Usui,Y. Determination of a new angiotensin converting enzyme inhibitor (CV-3317) and its metabolites in serum and urine by high-performance liquid chromatography, *Fukuoka.Igaku.Zasshi.*, **1985**, *76*, 441–450.

Delavirdine



Molecular formula: $C_{22}H_{28}N_6O_3S$, $C_{22}H_{28}N_6O_3S.CH_4O_3S$ (mesylate)
Molecular weight: 456.57
CAS Registry No.: 136817-59-9, 147221-93-0 (mesylate)
Merck Index: 2929

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Mix 100-200 μL plasma with 2 volumes of MeCN, centrifuge at 14000 rpm at 4° for 5 min, evaporate the supernatant to dryness, reconstitute the residue in 100 μL MeCN:100 mM pH 4 ammonium acetate buffer 10:90, inject a 75 μL aliquot. Urine. Centrifuge urine samples to remove large particulates, inject a 200 μL aliquot of the supernatant directly. Bile. Inject an aliquot directly.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC 5 μ-basic
Mobile phase: Gradient. A was MeCN. B was 100 mM pH 4 ammonium acetate buffer. A:B 10:90 for 5 min, to 60:40 over 35 min, maintain at 60:40 for 5 min
Flow rate: 1
Injection volume: 75
Detector: UV 295 (bile); Radioactivity, Radiomatic Model A-525. The column effluent mixed with Ultima-Flo M 1:3 and flowed to the detector (plasma, urine).

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

monkey; plasma

REFERENCE

Chang,M.; Sood,V.K.; Kloosterman,D.A.; Hauer,M.J.; Fagerness,P.E.; Sanders,P.E.; Vrbanac,J.J. Identification of the metabolites of the HIV-1 reverse transcriptase inhibitor delavirdine in monkeys, *Drug Metab.Dispos.*, **1997**, *25*, 814–827.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Condition a 200 mg C18 SPE cartridge with MeCN and 50 mM pH 7.4 K_3PO_4 buffer. Add 1250 μL MeCN to microsomal incubation and centrifuge. Add the supernatant to the SPE cartridge, wash with 2 mL water, elute with 500 μL MeCN.

HPLC VARIABLES

Column: 150 × 4.6 Zorbax SB-CN
Mobile phase: MeCN:50 mM pH 5.8 ammonium acetate containing 3% MeCN 26:74
Flow rate: 1.5
Detector: UV 295; F ex 305 em 425; Radioactivity, Radiomatic model A515, Packard Instrument Co., Meriden, with 500 μL cell, using Ultima-Flo M scintillant at ratio 1:3

CHROMATOGRAM

Retention time: 34.5

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

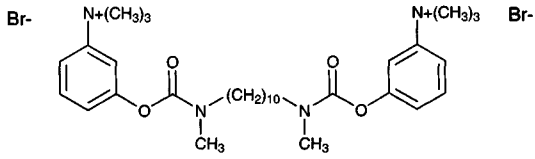
dog; human; liver; monkey; rat; SPE

REFERENCE

Voorman,R.L.; Maio,S.M.; Hauer,M.J.; Sanders,P.E.; Payne,N.A.; Ackland,M.J. Metabolism of delavirdine, a human immunodeficiency virus type-1 reverse transcriptase inhibitor, by microsomal cytochrome P450 in humans, rats, and other species: Probable involvement of CYP2D6 and CYP3A, *Drug Metab.Dispos.*, **1998**, *26*, 631–639.

Demecarium bromide

Molecular formula: $C_{32}H_{52}Br_2N_4O_4$
Molecular weight: 716.6
CAS Registry No.: 56-94-0
Merck Index: 2936



SAMPLE

Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeCN:buffer 50:50 (Buffer was 13.6 g/L sodium acetate, pH adjusted to 4.5 with glacial acetic acid.)
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: impurities, benzalkonium (C12, C14, C16), degradation products

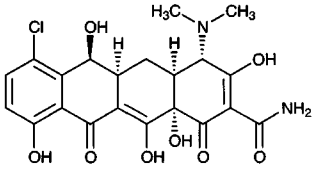
KEY WORDS

stability-indicating; ophthalmic solution

REFERENCE

Cohn,L.J.; Greely,V.J.; Tibbetts,D.L. Determination of demecarium bromide and related compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *321*, 401–405.

Demeclocycline



Molecular formula: $C_{21}H_{21}ClN_2O_8$
Molecular weight: 464.86
CAS Registry No.: 127-33-3, 64-73-3 (HCl), 13215-10-6 (sesquihydrate)
Merck Index: 2937

SAMPLE

Matrix: blood
Sample preparation: Add 50 μL 6% aqueous ascorbic acid solution, 1 mL phosphate-sulfite buffer, and 6 mL ethyl acetate to 250 μL plasma, vortex for 2 min, centrifuge at 2600 g for 10 min. Transfer 5 mL of the organic phase to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under nitrogen, redissolve the residue in 300 μL mobile phase, add 4 mL n-hexane, vortex, centrifuge at 2600 g for 5 min, remove the hexane layer by aspiration, inject a 100 μL aliquot of the aqueous phase. (The phosphate-sulfite buffer (pH 6) was 25.2 g sodium sulfite and 36.3 g NaH_2PO_4 dihydrate in 100 mL .)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher RP-18
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeCN:70% perchloric acid:water 29.85:0.25:69.9 containing 0.6 mM disodium EDTA and 5 mM oxalic acid, pH adjusted to 2.5 with 1 M NaOH
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 4.28
Internal standard: demeclocycline

OTHER SUBSTANCES

Extracted: doxycycline

KEY WORDS

plasma; turkey; demeclocycline is IS

REFERENCE

Santos,M.D.; Vermeersch,H.; Remon,J.P.; Schelkens,M.; De Backer,P.; Ducatelle,R.; Haesebrouck,F. Validation of a high-performance liquid chromatographic method for the determination of doxycycline in turkey plasma, *J.Chromatogr.B*, **1996**, *682*, 301–308.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 6% aqueous ascorbic acid + 400 μL buffer, vortex 30 s, add 3 mL ethyl acetate, vortex 5 min, centrifuge at 3000 rpm for 6 min. Remove organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH. Evaporate to dryness at 20° in a vortex evaporator, dissolve residue in 100 μL mobile phase, inject entire amount. (Buffer was 2 M NaH_2PO_4 and 2 M Na_2SO_3, pH 6.1.)

HPLC VARIABLES

Guard column: 4 μm Nova-Pak C18 (P/N 088141)
Column: 150 × 4.6 5 μm Ultrabase C18
Mobile phase: MeCN:water adjusted to pH 2.5 with phosphoric acid 28:72
Flow rate: 1
Injection volume: 100

Detector: UV 350

CHROMATOGRAM
Retention time: 2.7
Internal standard: demeclocycline

OTHER SUBSTANCES
Simultaneous: doxycycline

KEY WORDS
serum; demeclocycline is IS

REFERENCE
Gastearena,I.; Dios-Viéitez,M.C.; Segura,E.; Goñi,M.M.; Renedo,M.J.; Fos,D. Determination of doxycycline in small serum samples by liquid chromatography. Application to pharmacokinetical studies on small laboratory animals, *Chromatographia*, **1993**, *35*, 524–526.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 1 mL buffer, mix for 30 s, add 6 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. Urine. 100 μL Urine + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 400 μL buffer, mix for 30 s, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. (Buffer was 27.6 g NaH_2PO_4 + 25.2 g sodium sulfite in 100 mL water, pH 6.1.)

HPLC VARIABLES
Column: 100 × 2 5 μm Lichrosorb RP8
Mobile phase: MeCN:100 mM citric acid 24:76
Flow rate: 0.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 4
Internal standard: demeclocycline

OTHER SUBSTANCES
Extracted: oxytetracycline, tetracycline, methacycline, chlortetracycline, doxycycline

KEY WORDS
serum; demeclocycline is IS

REFERENCE
De Leenheer,A.P.; Nelis,H.J.C.F. Doxycycline determination in human serum and urine by high-performance liquid chromatography, *J.Pharm.Sci.*, **1979**, *68*, 999–1002.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk, capsules. Weigh out amount containing about 25 mg demeclocycline hydrochloride, dissolve in 25 mL 10 mM HCl, sonicate for 5 min, centrifuge at

2500 g for 5 min, filter (1.5 μm), inject an aliquot. Ointment. Weigh out ointment containing about 25 mg demeclocycline hydrochloride, add 25 mL hexane, add 25 mL 10 mM HCl, shake, filter the aqueous layer, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 8 μm 100 Å PLRP-S poly(styrene-divinylbenzene) (Polymer Labs)
Mobile phase: t-Butanol:200 mM pH 9.0 potassium phosphate buffer:20 mM pH 9.0 tetrabutylammonium hydrogen sulfate:10 mM pH 9.0 EDTA:water 6.8:10:15:10:58.2 (Flush with MeOH:water 50:50 at the end of each day.)
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Limit of quantitation: for impurities is 0.05% of demeclocycline

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

capsules; ointment

REFERENCE

Weng,N.D.; Roets,E.; Hoogmartens,J. Quantitative analysis of demeclocycline by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1691–1703.

SAMPLE

Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μg/mL solution in buffer, inject an aliquot. Capsules, tablets. Prepare a 1 mg/mL solution of capsule contents or crushed tablets in buffer, sonicate for 10 min, filter (0.45 μm), dilute with buffer, inject an aliquot. (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Laboratories)
Mobile phase: MeCN:buffer 25:75 (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

capsules; tablets

REFERENCE

Bryan,P.D.; Stewart,J.T. Chromatographic analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns with acidic mobile phases, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 675–692.

SAMPLE

Matrix: eggs, tissue

Sample preparation: Condition an Anagel-TSK Chelate-SPW column with 25 μL 50 mg/mL copper sulfate in water and 500 μL. Homogenize 2 g sliced chicken liver with 1.2 mL 1 M pH 4 citrate buffer and 12 mL ethyl acetate for 1 min. Homogenize 2 g sliced tissue with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 1 min. Shake 2 g blended egg with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 15 min. Centrifuge the mixture at 11000 rpm for 10 min, decant the supernatant, reextract the residue with two 12 mL portions of ethyl acetate. Add 10 g anhydrous sodium sulfate to the combined supernatants, swirl, let stand for 5-10 min, filter (Whatman 1PS phase-separating filter paper). Evaporate the filtrate to dryness or to an oily residue on a rotary evaporator under reduced pressure at 40°, reconstitute the residue in 2 mL MeOH by vortexing, filter (0.2 μm syringe filter). Add 1.5 mL of the filtrate to the Anagel column at 0.36 mL/min, wash with 500 μL water, 500 μL MeOH, and 500 μL water. Elute the contents of the Anagel column onto the analytical column with mobile phase A, after 11 min remove the Anagel column from the circuit, elute column B using gradient elution of mobile phase A:B, monitor the effluent from column B. (Prepare 1 M pH 4 or 5 citrate buffer as follows: dissolve 192 g citric acid in approximately 800 mL water, adjust pH value with 1 M NaOH and make up to 1 L with water.)

HPLC VARIABLES

Guard column: 5 × 3 PLRP-S

Column: 150 × 4.6 5 μm Polymer Labs PLRP-S

Mobile phase: Gradient. A:B 100:0 for 11 min, to 0:100 in 10 min, maintain at 0:100 for 10 min. A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid, and 10 mM EDTA).

Flow rate: 1

Injection volume: 1500

Detector: UV 350

CHROMATOGRAM

Retention time: 26

Limit of detection: 5 ng/g

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline, tetracycline

KEY WORDS

chicken; egg; metal chelate affinity chromatography; muscle; liver; salmon; trout; venison; SPE

REFERENCE

Cooper,A.D.; Stubbings,G.W.F.; Kelly,M.; Tarbin,J.A.; Farrington,W.H.H.; Shearer,G. Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products, *J.Chromatogr.A*, **1998**, *812*, 321–326.

SAMPLE

Matrix: formulations

Sample preparation: Dissolve ointment in petroleum ether, add an equal volume of EtOH:water 70:30, dilute with MeOH to 100 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm LiChrosorb Si-60

Mobile phase: MeOH:water 5:95 containing 1.3 mM disodium citrate, 1 mM tetrabutylammonium bromide, 1.1 mM citric acid, and 8 mM EDTA.

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.12

OTHER SUBSTANCES
Simultaneous: anhydrotetracycline, chlortetracycline, doxycycline, epianhydrotetracycline, oxytetracycline, quatrimycin, rolitetracycline, tetracycline

KEY WORDS
ointment

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: honey
Sample preparation: Condition a 500 mg Baker-10 C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 10 mL saturated aqueous disodium EDTA. Condition a 500 mg Baker-10 COOH cartridge with MeOH:ethyl acetate 10:90. Dissolve 25 g honey in 50 mL 100 mM pH 4.0 disodium EDTA-McIlvaine buffer, filter. Add the filtrate to the C18 SPE cartridge, wash with 20 mL water, wash with 400 μL ethyl acetate, air dry under vacuum for 5 min, elute with 50 mL MeOH:ethyl acetate 10:90. Add a 5 mL aliquot to the COOH SPE cartridge, wash with 5 mL MeOH (?), elute with 10 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Chemcosorb 3C8 (Chemco)
Mobile phase: MeCN:MeOH:10 mM aqueous oxalic acid 3:2:16, pH 3.0
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, methacycline, minocycline, oxytetracycline, tetracycline

KEY WORDS
SPE

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Suzuki,M. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey, *J.Chromatogr.*, **1987**, *400*, 253–261.

SAMPLE
Matrix: milk
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with

20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES

Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM

Retention time: 6.12

OTHER SUBSTANCES

Extracted: chlortetracycline, doxycycline, minocycline, oxytetracycline, tetracycline

KEY WORDS

metal chelate affinity chromatography; cow; SPE

REFERENCE

Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE

Matrix: milk
Sample preparation: Prepare a column as follows. Swirl Chelating Sepharose Fast Flow resin (Pharmacia) in its bottle, add it to a polypropylene column to give a bed volume of 1.0-1.2 mL, wash 3 times with 2 mL portions of water, wash with 2 mL 10 mM copper sulfate, wash with two 2 mL portions of water. Centrifuge 5 mL milk at 10° at 1500 g for 15 min, remove the lower layer and add it to 10 mL succinate buffer, mix, centrifuge at 1500 g for 30 min, add the supernatant to the column. Wash with 2 mL succinate buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, wash with 700 μL citrate/phosphate buffer (be careful not to disturb bed), elute with 2.5 mL citrate/phosphate buffer (column is white and eluate is blue). Filter (Amicon Centricon 30, MW 30000 cut-off; pre-washed by centrifuging with 2 mL water) while centrifuging at 5000 g for 30-90 min, inject a 600 μL aliquot of the ultrafiltrate. (Prepare succinate buffer by dissolving 11.8 g succinic acid in 980 mL water, adjust pH to 4.0 with 10 M NaOH, make up to 1 L. Prepare the citrate/phosphate buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 11 min, return to initial conditions.
Flow rate: 1
Injection volume: 600
Detector: UV 355

CHROMATOGRAM
Retention time: 14.2
Limit of detection: 1.02 ng/mL
Limit of quantitation: 2.18 ng/mL

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, methacycline, minocycline, oxytetracycline, tetracycline
Noninterfering: chloramphenicol, gentian violet, hydromycin B, ivermectin, spectinomycin, sulfa drugs

KEY WORDS
cow; SPE; ultrafiltrate

REFERENCE
Carson,M.C. Simultaneous determination of multiple tetracycline residues in milk using metal chelate affinity chromatography, *J.AOAC Int.*, **1993**, *76*, 329–334.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 5 μm 100 Å PLRP-S (Polymer Laboratories)
Mobile phase: MeCN:MeOH:buffer 15:10:60 (Buffer was 10 mM oxalic acid adjusted to pH 2.0 with 4 M HCl.)
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water.)

CHROMATOGRAM
Retention time: 8.3

OTHER SUBSTANCES
Simultaneous: chlortetracycline, oxytetracycline, tetracycline

KEY WORDS
protect from light; post-column reaction; derivatization; SPE; complexation

REFERENCE
Croubels,S.; Van Peteghem,C.; Baeyens,W. Sensitive spectrofluorimetric determination of tetracycline residues in bovine milk, *Analyst*, **1994**, *119*, 2713–2716.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond-Elut C8 SPE cartridge with 6 mL MeOH, 6 mL water, and 2 mL buffer A. Condition a 6 mL SPE cartridge containing 3 g wet XAD-2 resin with 10 mL MeOH, 10 mL water, and 2 mL buffer B. Homogenize (Ultra-Turrax) 2 g tissue with 20 mL succinate buffer for 1 min, centrifuge at 30 897 g for 15 min, filter (Whatman No. 1 paper) the supernatant, dilute 12 mL filtrate with 6 mL buffer B. For sheep liver add the diluted filtrate to the C8 SPE cartridge, wash with 10 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. For cow kidney add the diluted filtrate to the XAD-2 cartridge, wash with 14 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. Inject 25 μL 50 mg/mL copper sulfate and 500 μL water onto column

A then load 1.5 mL of the eluate from the SPE cartridge at 0.36 mL/min onto column A. Wash to waste with 500 μL water, 500 μL MeOH, and 500 μL water then elute the contents of column A onto column B with mobile phase A. After 11 min remove column A from the circuit and elute column B with a linear gradient of A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 10 min, re-equilibrate to 100:0. Monitor the effluent from column B. (Buffer A was 100 mM KH_2PO_4 containing 3 g/L pentanesulfonic acid. Succinate buffer was 60 g succinic acid in 1 L water adjusted to pH 4.0 with 1 M NaOH. Buffer B was 37.2 g disodium EDTA and 3 g pentanesulfonic acid in 1 L succinate buffer.)

HPLC VARIABLES

Column: A 10 × 6 10 μm Anagel-TSK-Chelate-SPW (Anachem); B 5 × 3 5 μm Polymer Labs. PLRP-S + 150 × 4.6 5 μm Polymer Labs. PLRP-S

Mobile phase: A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid and 10 mM EDTA.)

Injection volume: 1500

Detector: UV 350

CHROMATOGRAM

Retention time: 24

Limit of detection: 20 μg/kg

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline, tetracycline

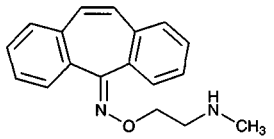
KEY WORDS

SPE; sheep; cattle; liver; kidney; column-switching

REFERENCE

Stubbings,G.; Tarbin,J.A.; Shearer,G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues, *J.Chromatogr.B*, **1996**, *679*, 137–145.

Demexiptiline



Molecular formula: $C_{18}H_{18}N_2O$
Molecular weight: 278.35
CAS Registry No.: 24701-51-7, 18059-99-9 (HCl)
Merck Index: 2941

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 6.52
Limit of detection: <120 ng/mL

KEY WORDS

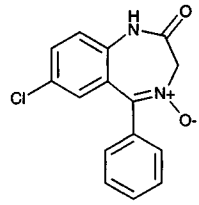
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Demoxepam



Molecular formula: $C_{15}H_{11}ClN_2O_2$
Molecular weight: 286.72
CAS Registry No.: 963-39-3
Lednicer No.: 2 401

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL MeOH, shake, centrifuge at 7000 g for 7 min, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:100 mM pH 8 phosphate buffer 60:40
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 410 following post-column photolysis. The column effluent was mixed with bubbles (at 0.7 mL/min) and flowed through a 3.8 m × 1.1 mm ID PTFE coil irradiated by a mercury-xenon lamp to a debubbler, the liquid phase then flowed to the detector. (The air bubbles suppress band broadening in the PTFE coil.)

CHROMATOGRAM

Retention time: 8
Limit of detection: 100 pg

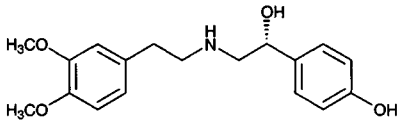
KEY WORDS

serum; post-column reaction; post-column photochemical derivatization

REFERENCE

Brinkman,U.A.T.; Welling,P.L.M.; De Vries,G.; Scholten,A.H.M.T.; Frei,R.W. Liquid chromatography of demoxepam and phenothiazines using a post-column photochemical reactor and fluorescence detection, *J.Chromatogr.*, **1981**, *217*, 463–471.

Denopamine



Molecular formula: $C_{18}H_{23}NO_4$
Molecular weight: 317.38
CAS Registry No.: 7171-90-9
Merck Index: 2943

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M K_2HPO_4 + 10 mL chloroform, shake mechanically for 15 min, centrifuge at 2000 g for 10 min. Remove 8 mL of the organic layer and add it to 9 mL 100 mM HCl, shake for 15 min, centrifuge at 2000 g for 15 min. Remove 8 mL of the aqueous phase and add it to 1.5 mL 500 mM sodium bicarbonate containing 50 mM NaOH (to make it weakly alkaline), add to a 100 mg C18 Bond Elut SPE cartridge, wash with 300 µL MeOH:100 mM HCl 25:75, elute with 300 µL EtOH: concentrated HCl 99.91:0.09. Evaporate the eluate to dryness, reconstitute in 300 µL 125 ng/mL phenolphthalein in mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Nucleosil 5C18
Mobile phase: MeCN:100 mM K_2HPO_4 27:100, pH adjusted to 5.5 with orthophosphoric acid
Column temperature: 25
Flow rate: 0.9
Injection volume: 100
Detector: E, Yanagimoto Model VMD 501, +750 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 10
Internal standard: phenolphthalein (17)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Noninterfering: digoxin, enalapril, captopril, furosemide, nifedipine, diltiazem, nitroglycerin, metabolites

KEY WORDS

plasma; SPE; dog; pharmacokinetics

REFERENCE

Tagawa,K.; Ueki,T.; Mizobe,M.; Noda,K.; Samejima,M. Determination of denopamine in human and dog plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1990**, *529*, 500–506.

SAMPLE

Matrix: formulations
Sample preparation: Grind tablets, weigh out amount equivalent to 10 mg denopamine, add 20 mL mobile phase, shake vigorously for 5 min, filter (0.45 µm). Dilute a 5 mL aliquot of the filtrate to 20 mL with mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 6 5 µm ULTRON ES-PhCD (Shinwakako)
Mobile phase: MeCN:50 mM pH 4.6 NaH_2PO_4 25:75
Column temperature: 40
Flow rate: 1

Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.95 (S-(+)), 8.11 (R-(-))
Limit of detection: 0.1% (of major enantiomer)

KEY WORDS
tablets; chiral

REFERENCE
Nishi,H.; Ishibuchi,K.; Nakamura,K.; Nakai,H.; Sato,T. Enantiomeric separation of denopamine by capillary electrophoresis and high-performance liquid chromatography using cyclodextrins, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1483–1492.

SAMPLE
Matrix: solutions

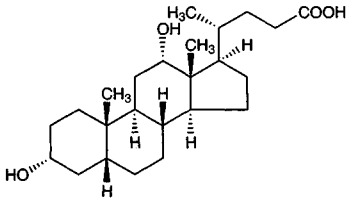
HPLC VARIABLES
Column: 150 × 6 5 μm Ultron ES-Ph-CD immobilized β-cyclodextrin (Shinwakako)
Mobile phase: MeCN:50 mM pH 4.6 phosphate buffer 30:70
Column temperature: 40
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 7 (S-(+)), 8.3 (R-(-))

KEY WORDS
chiral

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T.; Terabe,S. Enantiomeric separation of trimetoquinol, denopamine and timepidium by capillary electrophoresis and HPLC and the application of capillary electrophoresis to the optical purity testing of the drugs, *Chromatographia*, **1995**, *40*, 638–644.

Deoxycholic acid



Molecular formula: $C_{24}H_{40}O_4$
Molecular weight: 392.58
CAS Registry No.: 83-44-3, 302-95-4 (sodium salt)
Merck Index: 2946

SAMPLE

Matrix: bile

Sample preparation: 200 μL Bile + 4 M NaOH:MeOH 50:50, heat at 80° for 16 h, adjust pH to 1.5 with 6 M HCl, extract three times with 10 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dry dichloromethane, add 1 mg p-aminophenol, add 150 μL triethylamine, add at least a 3-fold molar excess of 2-bromo-1-methylpyridinium iodide, heat at 60° for 30 min, cool, concentrate under a stream of nitrogen, add 1 mL 100 mM HCl, add 1 mL ethyl acetate, shake vigorously, centrifuge at 2500 rpm for 5 min, inject an aliquot of the supernatant. (Prepare 2-bromo-1-methylpyridinium iodide by analogy with the preparation of 2-chloro-1-methylpyridinium iodide. Add 15 g methyl iodide to 13.9 g 2-bromopyridine in 3 mL acetone at 0°, stir at room temperature for 3 days. Filter the precipitate and wash it with 50 mL dry ether, dry under reduced pressure to give 2-bromo-1-methylpyridinium iodide (Bull. Chem. Soc. Japan 1977, 50, 1863).)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C-18
Mobile phase: MeOH:water:perchloric acid 75:25:0.1 containing 50 mM sodium perchlorate
Column temperature: 25 ± 0.1
Flow rate: 0.9
Detector: E, 0.75 v, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 17

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, lithocholic acid

KEY WORDS

derivatization

REFERENCE

Ikenoya,S.; Hiroshima,O.; Ohmae,M.; Kawabe,K. Electrochemical detector for high performance liquid chromatography. IV. Analysis of fatty acids, bile acids and prostaglandins by derivatization to an electrochemically active form, *Chem.Pharm.Bull.(Tokyo)*, **1980**, *28*, 2941–2947.

SAMPLE

Matrix: bile

Sample preparation: Extract bile with 20 volumes EtOH, boil on a hot water bath, cool, let stand overnight, filter (Toyo Roshi 5A paper), filter (0.45 μm), add 200 μg/mL testosterone acetate in EtOH (final IS concentration 100 μg/mL), inject a 5-10 μL aliquot.

HPLC VARIABLES

Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:30 mM phosphate buffer 10:60:30, pH 3.40
Flow rate: 0.5
Injection volume: 5-10

Detector: UV 200

CHROMATOGRAM

Retention time: 27 (taurine conjugate), 33 (glycine conjugate)
Internal standard: testosterone acetate (39)
Limit of detection: 50 ng

OTHER SUBSTANCES

Extracted: ursodiol, conjugates, bile acids, chenodiol

REFERENCE

Nakayama,F.; Nakagaki,M. Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 287–293.

SAMPLE

Matrix: bile
Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. 50 μL Bile + 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge (J. Pharm. Biomed. Anal. 1990, 8, 235). Evaporate to dryness under a stream of nitrogen, reconstitute with 2 mL MeOH, sonicate at 40° for 3 min, filter (0.2 μm). Add a 500 μL aliquot of the filtrate to 50 μL 0.01% KOH in MeOH, evaporate to dryness, reconstitute with 200 μL MeOH:water 10:90, sonicate at 40° for 3 min, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil RP-18
Mobile phase: Gradient. MeCN:water 60:40 for 10 min, to 80:20 over 10 min, maintain at 80:20 for 25 min, return to initial conditions over 5 min.
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 30
Internal standard: 6-methoxynaphthacyl ester of valproic acid (23)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; SPE

REFERENCE

Cavrini,V.; Gatti,R.; Roda,A.; Cerrè,C.; Roveri,P. HPLC-fluorescence determination of bile acids in pharmaceuticals and bile after derivatization with 2-bromoacetyl-6-methoxynaphthalene, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 761–770.

SAMPLE

Matrix: bile, blood

Sample preparation: Serum. 100-200 μL Serum + 1 mL MeOH, mix, sonicate for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute with 1 mL 50 mM pH 7.0 phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot. Bile. Mix 10 μL bile with 10 mL 50 mM pH 7.0 phosphate buffer, add a 1 mL aliquot to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 μm Model RCM-100 Radial-Pak A (Waters)

Mobile phase: Gradient. MeCN:MeOH:water 100:50:40 for 30 min then 100:50:20 (step gradient).

Flow rate: 2

Injection volume: 8

Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 45

Internal standard: lauric acid (56)

Limit of detection: 10 pmole

Limit of quantitation: 50 pmole

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS

derivatization; serum; SPE

REFERENCE

Kamada,S.; Maeda,M.; Tsuji,A. Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, *J.Chromatogr.*, **1983**, *272*, 29–41.

SAMPLE

Matrix: bile, blood, feces, gastric contents, tissue

Sample preparation: Condition a Sep-Pak C18 cartridge with 2 mL 720 mM MeOH in water and 6 mL 100 mM pH 7.0 potassium phosphate buffer. Serum. 200 μL Serum + 1 mL MeCN, mix, sonicate for 10 min, centrifuge at 17000 g for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE

cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Liver. Homogenize (glass homogenizer) liver in 1 mL 720 mM EtOH in water, add 2 mL 720 mM EtOH in water, heat at 75° for 15 min, centrifuge at 17000 g for 10 min, remove the supernatant, extract the residue twice more. Combine the supernatants and evaporate them to dryness at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Bile. Dilute 20 μL bile with 10 mL 100 mM pH 7.0 potassium phosphate buffer. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Gastric juice. Dilute 1 mL gastric juice with 9 mL 100 mM pH 7.0 potassium phosphate buffer, sonicate for 10 min. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Feces. Dilute 1 g feces with 9 mL MeOH, mix thoroughly, sonicate for 10 min, centrifuge at 17000 g for 10 min. Remove a 1 mL aliquot of the supernatant and evaporate it to dryness, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere I.P. C18

Mobile phase: Gradient. A was MeCN:MeOH:water 100:50:75. B was MeCN:MeOH 100:50. A:B 100:0 for 7 min, to 70:30 over 0.5 min, maintain at 70:30 for 5 min, to 50:50 over 0.5 min, maintain at 50:50 over 7 min, to 25:75 over 1 min, maintain at 25:75 for 7 min.

Column temperature: 35

Flow rate: 1.7

Injection volume: 100

Detector: F

CHROMATOGRAM

Retention time: 22

Internal standard: lauric acid (24.5)

Limit of detection: 0.5 pmole

OTHER SUBSTANCES

Extracted: chenodiol (chenodeoxycholic acid), cholic acid, glycinechenodeoxycholic acid, glycinecholic acid, glycinedeoxycholic acid, glycinelithocholic acid, glycineursodeoxycholic acid, lithocholic acid, ursodiol (ursodeoxycholic acid)

KEY WORDS
derivatization; SPE; liver; serum

REFERENCE
Güldütuna,S.; You,T.; Kurts,W.; Leuschner,U. High performance liquid chromatographic determination of free and conjugated bile acids in serum, liver biopsies, bile, gastric juice and feces by fluorescence labeling, *Clin.Chim.Acta*, **1993**, *214*, 195–207.

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Urine. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-200 μL urine 1:4 with 100 mM NaOH, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Serum. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-500 μL serum with 3.5 mL 100 mM NaOH, heat at 64° for 30 min, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Bile. Dilute 1:500 to 1:1000 with mobile phase, filter (0.22 μm, inject an aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL C18
Mobile phase: MeOH:15 mM ammonium acetate 80:20, apparent pH 6.0 ± 0.1
Flow rate: 0.3
Detector: MS, electrospray, Fisons VG TRIO 2000 quadrupole (6% of the mobile phase was diverted to the MS detector) or evaporative light scattering detector (Varex)

CHROMATOGRAM
Retention time: 19.15
Limit of detection: 15 pg

OTHER SUBSTANCES
Extracted: chenodiol, ursodiol, bile acids, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycoursodeoxycholic acid, lithocholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid

KEY WORDS
serum; SPE; hamster; human; LC-MS

REFERENCE
Roda,A.; Gioacchini,A.M.; Cerrè,C.; Baraldini,M. High-performance liquid chromatographic-electrospray mass spectrometric analysis of bile acids in biological fluids, *J.Chromatogr.B*, **1995**, *665*, 281–294.

SAMPLE
Matrix: bile, gastric contents
Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. Mix 50 μL bile or 500 μL gastric juice with 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH: 40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge. Evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL initial mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH:30 mM sodium acetate 65:35, adjusted to pH 4.3 with phosphoric acid. B was MeOH:70 mM sodium acetate 90:10, adjusted to pH 4.3 with phosphoric acid. A:B 85:15 for 10 min, to 10:90 over 25 min, maintain at 10:90 for 5 min.

Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Extracted: cholic acid, chenodiol, lithocholic acid, ursodiol

KEY WORDS
SPE

REFERENCE
Scalia,S. Group separation of free and conjugated bile acids by pre-packed anion-exchange cartridges, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH, 10 mL water, and 2 mL 100 mM pH 8.0 Tris-HCl buffer. 5-7 mL Serum + 19 volumes 100 mM pH 8.0 Tris-HCl buffer, sonicate for 10 min, add to the SPE cartridge, wash with 15 mL water, elute with 6-7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, dissolve residue in water, filter (Millipore GS 0.22 μm), wash filter, evaporate filtrates to dryness, reconstitute in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:20 mM KH_2PO_4 65:35, adjust pH to 5.3
Flow rate: 1.4
Injection volume: 30
Detector: UV 210

CHROMATOGRAM
Retention time: 18 (taurine conjugate), 24 (glycine conjugate)
Limit of detection: 40 nM (glycine conjugate), 30 nM (taurine conjugate)

OTHER SUBSTANCES
Extracted: conjugates, ursodiol, chenodiol, bile acids

KEY WORDS
serum; SPE

REFERENCE
Linnet,K. A high-pressure liquid chromatogaphic-enzymatic assay for glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acid in serum, *Scand.J.Clin.Lab.Invest.*, **1982**, *42*, 455–460.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SPE cartridge with 5 mL EtOH and 5 mL water. 100 μL Serum + 250 ng deoxycholic acid 12-propionate + 1 mL 500 mM pH 7.0 phosphate buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL 1.5% EtOH, elute with 2 mL 90% EtOH. Evaporate a 400 μL aliquot of the eluate, add 100 μL 2 mg/mL 1-anthroyl nitrile in MeCN, add 0.16% quinuclidine in MeCN, heat at 60° for 20 min, add 50 μL MeOH, evaporate under nitrogen. Dissolve the residue in 1 mL 90% EtOH, add to a 18 × 6 100 mg column of PHP-LH-20 Sephadex at 0.2 mL/min, wash with 1 mL 90% EtOH, elute with 5 mL 100 mM acetic acid in 90% EtOH (free bile acids),

elute with 5 mL 200 mM formic acid in 90% EtOH (glycine-conjugated bile acids), elute with 5 mL 300 mM pH 6.3 acetic acid-potassium acetate in 90% EtOH (taurine-conjugated bile acids). Evaporate each fraction, dissolve the residue in 100-200 μL MeOH, inject a 5-10 μL aliquot. (Preparation of PHP-LH-20 Sephadex is as follows. Suspend 75.7 g Sephadex LH-20 in 200 mL dichloromethane using a glass stirring rod (not a magnetic stirrer) for 30 min, add 19 mL boron trifluoride ethyl etherate, after 15 min add 50 mL 35% epichlorohydrin in dichloromethane at 1-2 mL/min (Caution! Epichlorohydrin is a carcinogen!), stir for another 30 min, filter, wash with EtOH, dry chlorohydroxypropyl Sephadex LH-20 at 50° (J.Chromatogr. 1971, 59, 45). Stir 27.2 g chlorohydroxypropyl Sephadex LH-20 in 100.5 mL piperidine at room temperature for 30 min, add 5.74 g KOH in 302 mL MeOH, heat at 50-60° for 3 h with occasional shaking, filter, wash with EtOH:water 50:50, wash with 200 mM acetic acid in EtOH:water 70:30, wash with EtOH:water 90:10 until washings become neutral, store in EtOH:water 90:10 (Clin. Chim. Acta 1978 87 141). Prepare 1-anthroyl nitrile as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroyl nitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18
Mobile phase: MeOH:0.3% pH 6.0 potassium phosphate buffer 5:1
Flow rate: 1.8
Injection volume: 10
Detector: F ex 370 em 470

CHROMATOGRAM

Retention time: 15
Internal standard: deoxycholic acid 12-propionate (20)
Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, ursodiol

KEY WORDS

serum; SPE; derivatization

REFERENCE

Goto,J.; Saito,M.; Chikai,T.; Goto,N.; Nambara,T. Studies on Steroids. CLXXXVII. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling, *J.Chromatogr.*, **1983**, *276*, 289–300.

SAMPLE

Matrix: blood
Sample preparation: Deproteinize 20 μL serum with a pretreatment column (Autoserumout, Sekisui), inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Medipola Bile column (Sekisui)

Mobile phase: Gradient. A was MeCN:MeOH:30 mM ammonium acetate 20:20:60. B was MeCN:MeOH:30 mM ammonium acetate 30:30:40. A:B from 100:0 to 80:20 over 10 min, to 0:100 over 27 min, maintain at 0:100 for 30 min.

Flow rate: 1

Detector: F ex 340 em 460 following post-column reaction detection. The effluent from the column was mixed with reagent pumped at 1 mL/min, the mixture flowed through a 20 × 4 3α-HSD column (Sekisui) containing bound 3α-hydroxysteroid dehydrogenase to the detector. (The reagent was 1.36 g/L KH_2PO_4, 372 mg/L disodium EDTA, 140 mg/L βNAD, and 450 μL/L 2-mercaptoethanol in water adjusted to pH 7.8 with 5 M KOH.)

CHROMATOGRAM

Retention time: 37

OTHER SUBSTANCES

Extracted: ursodiol, chenodiol, bile acids

KEY WORDS

post-column reaction; immobilized enzyme reactor; serum

REFERENCE

Adachi,Y.; Nanno,T.; Itoh,T.; Kurumi,Y.; Yamazaki,K.; Sawada,Y.; Yamamoto,T. Determination of individual serum bile acids in chronic liver diseases: fasting levels and results of oral chenodeoxycholic acid tolerance test, *Gastroenterol.Jpn.*, **1988**, *23*, 401–407.

SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Dilute 100-200 μL serum with 4 mL 400 mM sodium bicarbonate, add to the SPE cartridge, wash with 20 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, add 400 μg sodium carbonate, add 50 μL 20 mg/mL 18-crown-6 in MeCN, heat at 40° for 1 h, make up to 500 μL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Nova-Pak ODS

Mobile phase: Gradient. A was MeCN:MeOH:water 15:13.8:71.2. B was MeCN. A:B from 100:0 to 37:63 over 47 min (Waters convex curve + 2), to 0:100 over 0.1 min (Waters curve +9), maintain at 0:100 for 7.9 min, re-equilibrate at initial conditions for 6 min.

Flow rate: 1 for 47 min then 1.5

Injection volume: 10

Detector: F ex 320 em 385

CHROMATOGRAM

Retention time: 46.47

Limit of detection: 80 nM

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, ursodiol

KEY WORDS

derivatization; serum; SPE

REFERENCE
Wang,G.F.; Stacey,N.H.; Earl,J. Determination of individual bile acids in serum by high performance liquid chromatography, *Biomed.Chromatogr.*, **1990**, *4*, 136–140.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1-5 mg bile acid in 500 μL chloroform with enough MeOH to make a solution, add a solution of 1-naphthyldiazomethane in ether until the reddish-orange color persists, if the color disappears within 1 h add more reagent, add 1 drop acetic acid to decompose excess reagent, make up to 1 mL, inject a 5 μL aliquot. (Preparation of 1-naphthyldiazomethane is as follows. Stir 6.7 g 1-naphthaldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h (Caution! Hydrazine hydrate is a carcinogen!). Remove the solid by filtration and recrystallize it twice from EtOH to give 1-naphthaldehyde hydrazone as white crystals (mp 91-92°). Stir 3.1 g 1-naphthaldehyde hydrazone, 5 g anhydrous sodium sulfate, 50 mL ether, 1 mL EtOH saturated with KOH, and 10 g yellow mercuric oxide for 5 h, filter (sintered glass), concentrate the filtrate under reduced pressure to give 1-naphthyldiazomethane as red crystals (mp 40-41°) (Bull. Chem. Soc. Japan 1967, 40, 691).)

HPLC VARIABLES
Column: 300 mm long μPorasil
Mobile phase: Hexane:THF:MeOH 75:30:2
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 10
Limit of detection: 20-30 ng

OTHER SUBSTANCES
Simultaneous: chenodiol, 3,7-dihydroxy-12-ketocholanic acid, lithocholic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Matthees,D.P.; Purdy,W.C. Naphthyldiazomethane as a derivatizing agent for the high-performance liquid chromatography detection of bile acids, *Anal.Chim.Acta*, **1979**, *109*, 161–164.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of solution (or hydrolyzed bile) with a 50% molar excess of triethylamine in MeCN, warm briefly, add a 50% molar excess of 100 mM 2-bromoacetophenone in MeCN, heat at 80-90° for 45-60 min, evaporate to dryness, reconstitute with dioxane (Caution! Dioxane is a carcinogen!), filter (0.47 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10/25 ODS
Mobile phase: Gradient. n-Heptane:dioxane 90:10 for 3 min then n-heptane:dioxane:isopropanol 70:25:5 (step gradient). (Caution! Dioxane is a carcinogen!)
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 17
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, hyodeoxycholic acid, lithocholic acid, ursodiol

KEY WORDS
derivatization

REFERENCE
Stellaard,F.; Hachey,D.L.; Klein,P.D. Separation of bile acids as their phenacyl esters by high-pressure liquid chromatography, *Anal.Biochem.*, **1978**, *87*, 359–366.

SAMPLE
Matrix: solutions
Sample preparation: Treat a solution in MeOH with a slight excess of tetramethylammonium hydroxide in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with MeCN, add a 2-10 fold excess of 9-(chloromethyl)anthracene in cyclohexane, heat at 75° for 15 min, very dilute solutions may require longer times), dilute with MeCN, inject an aliquot.

HPLC VARIABLES
Column: 300 mm long "Fatty Acid" reversed-phase (Waters)
Mobile phase: MeOH:water 88:12 (A) or 82:18 (B)
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Simultaneous: cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid
Interfering: chenodiol

KEY WORDS
derivatization

REFERENCE
Korte,W.D. 9-(Chloromethyl)anthracene: a useful derivatizing reagent for enhanced ultraviolet and fluorescence detection of carboxylic acids with liquid chromatography, *J.Chromatogr.*, **1982**, *243*, 153–157.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water. Treat a bile salt solution with 3α- or 7α-hydroxysteroid dehydrogenase and NAD at 37° for 1 h, add 4 drops concentrated HCl, add to the SPE cartridge, wash with 10 mL water, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL 650 μL/L concentrated HCl in EtOH and 200 μL 1 mg/mL dansylhydrazine in EtOH, heat at 60° for 10 min, inject an aliquot. (Hydroxy groups are oxidized to ketones which are then derivatized.)

HPLC VARIABLES
Column: 10 μm Radial-Pak C18 (8 mm ID, Waters)
Mobile phase: Gradient. MeOH:10 mM pH 3.4 KH_2PO_4 buffer from 60:40 to 85:15 over 13 min.
Flow rate: 2
Detector: F ex 360 em 510

CHROMATOGRAM
Retention time: 18
Limit of quantitation: 30 pmole

OTHER SUBSTANCES
Simultaneous: cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, lithocholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, taurolithocholic acid, ursodeoxycholic acid
Interfering: chenodeoxycholic acid

KEY WORDS
derivatization; SPE; comparison with 2,4-dinitrophenylhydrazine derivatization

REFERENCE
Reid,A.D.; Baker,P.R. Formation and separation by reversed-phase high-performance liquid chromatography of fluorescent and UV-absorbing bile salt derivatives, *J.Chromatogr.*, **1983**, *260*, 115–121.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 100 μL aliquot of a 1-100 μM solution in 20 mM pH 7.1 phosphate buffer with 10 μL 10 mM tetrabutylammonium hydrogen sulfate in water and 100 μL 1 mM N-(9-acridinyl)bromoacetamide in chloroform, stir at 90° for 20-30 min, cool. Remove a 10 μL aliquot of the organic layer and evaporate it to dryness under reduced pressure, reconstitute with mobile phase, sonicate, inject an aliquot. (Synthesis of N-(9-acridinyl)bromoacetamide is as follows. Dissolve 2.49 g 9-aminoacridine hydrochloride hydrate in water, add dilute NaOH to precipitate the free base, extract with ethyl acetate, dry over anhydrous magnesium sulfate, filter, evaporate to give 9-aminoacridine as yellow needle-shaped crystals (mp 239-240°). Add 1.01 g bromoacetyl bromide in 20 mL diethyl ether dropwise with stirring to 970 mg 9-aminoacridine dissolved in 50 mL acetone containing 1.02 g triethylamine, filter, wash the solid with acetone. Evaporate the filtrate and chromatograph the residue on a 260 × 30 glass column of 70-230 mesh silica gel 60 (Merck) with chloroform:ethyl acetate 2:1. Collect the strong yellow band and evaporate it to dryness, recrystallize from MeOH to give N-(9-acridinyl)bromoacetamide as light yellow crystals (mp 180-182° d).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:water:phosphoric acid 40:60:0.2
Flow rate: 1
Injection volume: 20
Detector: F ex 357.5 em 482

CHROMATOGRAM
Retention time: 32
Limit of detection: 10 fmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid

KEY WORDS
derivatization

REFERENCE
Allenmark,S.; Chelminska-Bertilsson,M.; Thompson,R.A. N-(9-Acridinyl)-bromoacetamide -A powerful reagent for phase-transfer-catalyzed fluorescence labeling of carboxylic acids for liquid chromatography, *Anal.Biochem.*, **1990**, *185*, 279–285.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100-500 μL of a solution in MeCN with 300 μL 1.28 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN, add 50 μL 3% triethylamine in MeCN, heat at 70° for 30 min, cool, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil RP-18
Mobile phase: Gradient. MeCN:water from 55:45 to 80:20 over 20 min, maintain at 80:20 for 10 min, return to initial conditions over 10 min.
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 19.5
Limit of detection: 2-3 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, glycochenodeoxycholic acid, glycocholic acid, lithocholic acid

KEY WORDS
derivatization

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P. 2-Bromoacetyl-6-methoxynaphthalene: A useful fluorescent labelling reagent for HPLC analysis of carboxylic acids, *Chromatographia*, **1992**, *33*, 13–18.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:0.8 M NaOH 8:92, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: CarboPac PA-100 (Dionex)
Column: 250 × 4 8.5 μm CarboPac PA-100 (Dionex)
Mobile phase: MeCN:water 15:85 containing 900 mM sodium acetate and 100 mM NaOH
Flow rate: 0.8
Injection volume: 25
Detector: E, Dionex PAD-2 pulsed amperometric detector, gold working electrode, V1 + 0.05 V, t1 480 ms, V2 + 0.60 V, t2 120 ms, V3 -0.60 V, t3 60 ms

CHROMATOGRAM
Retention time: 5.38

OTHER SUBSTANCES
Simultaneous: chenodiol, ursodiol, cholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, glycodeoxychenodeoxycholic acid, ursodeoxycholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, glycolithocholic acid, lithocholic acid, taurolithocholic acid

REFERENCE
Chaplin,M.F. Analysis of bile acids and their conjugates using high-pH anion-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.B*, **1995**, *664*, 431–434.

SAMPLE
Matrix: solutions
Sample preparation: Mix 200 μL of a solution of bile acids with 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, add 300 μL 10 mM tetrakis(decyl)ammonium bromide in 100 mM pH 7.0 phosphate buffer, heat at 40° for with sonication 10 min, add 300 μL 5.1 μM IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES
Column: 250 × 4.6 Ultracarb 5 ODS
Mobile phase: Gradient. A was water. B was MeCN:MeOH 60:40. A:B 55:45 for 20 min, to 30:70 over 10 min, maintain at 30:70 for 25 min, return to initial conditions over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 40
Internal standard: 6-methoxynaphthacyl ester of lauric acid (36)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, lithocholic acid, ursodiol

KEY WORDS
derivatization

REFERENCE
Gatti,R.; Roda,A.; Cerre,C.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum, *Biomed.Chromatogr.*, **1997**, *11*, 11–15.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, pass 40 mL urine through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 10 mL 100 mM pH 5.0 sodium acetate buffer, add 100 μg β-glucuronidase, add 100 μg cholylglycine hydrolase, heat at 37° for 36 h, pass the mixture through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 85 μm μBondapak C18 radial pack
Mobile phase: MeCN:MeOH:water:acetic acid 70:20:70:1
Flow rate: 2
Injection volume: 50
Detector: RI

CHROMATOGRAM

Retention time: 37
Limit of detection: 1000 ng

OTHER SUBSTANCES

Extracted: ursodiol, bile acids, chenodiol

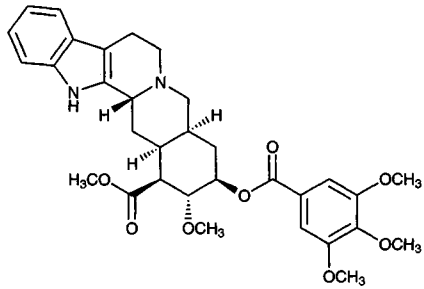
KEY WORDS

SPE

REFERENCE

Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, *26*, 690–698.

Deserpidine



Molecular formula: $C_{32}H_{38}N_2O_8$
Molecular weight: 578.66
CAS Registry No.: 131-01-1
Merck Index: 2964
Lednicer No.: 1 320

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen,

procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5

OTHER SUBSTANCES

Simultaneous: triflupromazine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, thiothixene, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

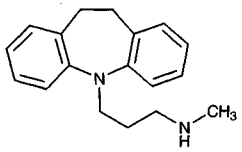
KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

Desipramine



Molecular formula: $C_{18}H_{22}N_2$
Molecular weight: 266.39
CAS Registry No.: 50-47-5, 58-28-6 (HCl)
Merck Index: 2966
Lednicer No.: 1 402

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM

Retention time: 8.92
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, clomipramine, fluoxetine, imipramine, maprotiline, nortriptyline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid
Interfering: desmethylmaprotiline

KEY WORDS

plasma

REFERENCE

Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE

Matrix: blood
Sample preparation: 1.0 mL Plasma + 100 μL 50 mM pH 7.5 Na_2HPO_4/K_2HPO_4, vortex briefly. Add 5 mL diethyl ether and shake horizontally at 100 rpm for 20 min. Centrifuge at 3000 rpm for 10 min, snap-freeze in a dry-ice/EtOH bath. Add 100 μL 50 mM HCl to the decanted organic phase, vortex for 60 s, snap-freeze, decant organic phase to waste. Inject a 50 μL aliquot of the acid extract.

HPLC VARIABLES
Column: 100 × 4 5 μm Lichrocart RP-SelectB
Mobile phase: MeCN: 40 mM pH 5.5 Na2HPO4 buffer 25:75
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 18.9
Internal standard: desipramine

OTHER SUBSTANCES
Extracted: diltiazem

KEY WORDS
plasma; desipramine is IS

REFERENCE
Morris,R.G.; Saccoia,N.C.; Jones,T.E. Modified liquid chromatographic assay for diltiazem and metabolites in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2385–2394.

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. Add 500 μL plasma to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 μL MeOH, vortex, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS/CN
Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30
Flow rate: 1
Injection volume: 25
Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

CHROMATOGRAM
Retention time: 11.5
Internal standard: desipramine

OTHER SUBSTANCES
Extracted: paroxetine, venlafaxine

KEY WORDS
plasma; SPE; desipramine is IS

REFERENCE
Clement,E.M.; Odontiadis,J.; Franklin,M. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction, *J.Chromatogr.B*, **1998**, *705*, 303–308.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Plasma + 1600 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: μBondapak/Porasil
Column: μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: clomipramine (7.5)
Limit of detection: 0.5 ng

OTHER SUBSTANCES

Extracted: amitriptyline, imipramine, nortriptyline
Simultaneous: chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, diazepam, doxepin, flurazepam, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine

KEY WORDS

plasma

REFERENCE

Wong,S.H.Y.; McCauley,T. Reversed phase high-performance liquid chromatographic analysis of tricyclic antidepessants in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 849–862.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 200 μL 10 μg/mL protriptyline in water + 200 μL 80 g/L $NaHCO_3$ + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100 μL mobile phase, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 9.23
Internal standard: protriptyline (12.20)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: imipramine, trimipramine, doxepin, desmethyldoxepin, amitriptyline, nortriptyline, chlorpromazine, thioridazine, propranolol, propoxyphene, maprotiline, procainamide, disopyramide

Noninterfering: caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen, trifluoperazine

KEY WORDS
serum

REFERENCE
Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then 200 μL 700 ng/mL promazine in MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200 μL 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200 μL mobile phase, vortex 10 s, inject 75-100 μL aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25 μL concentrated HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 9.5
Internal standard: promazine (5.2)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desmethyldoxepin, doxepin, imipramine, nortriptyline, protriptyline
Simultaneous: thioridazine, hydroxyamoxapine, meperidine, chlorpromazine, disopyramide, amphetamine, 2-hydroxyimipramine, iprindole, pyrilamine, promethazine, prolixin, amoxapine, N-acetylprocainamide, procainamide, zimeldine, morphine, codeine, trifluoperazine, desmethyldisopyramide, 10-hydroxynortriptyline, prochlorperazine, oxaprotiline, 2-hydroxydesipramine, chlorpheniramine, maprotiline, norzimeldine, iminostilbene, desmethylchlordiazepoxide, buprion, diazepam, demoxepam, chlordiazepoxide, propoxyphene, dextropropoxyphene, cocaine, oxapam, trimipramine, mianserin, trimeprazine, loxepin, fluphenazine, methadone, trifluopromazine, phenteramine, chlorimipramine, perphenazine, quinidine
Noninterfering: thiopropazine

KEY WORDS
serum; normal phase

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane:isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.95
Internal standard: loxapine (k' 7.18)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, haloperidol, imipramine, nortriptyline, oxazepam, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine

KEY WORDS

plasma

REFERENCE

Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum or plasma + 0.6 mL water, vortex, extract with 10 mL toluene:isoamyl alcohol 99:1 for 10 min on a rotator, centrifuge for 5 min. Remove upper organic layer, evaporate under a stream of nitrogen at 37°, take up in 150 μL mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 μL aliquot of the lower layer.

HPLC VARIABLES

Column: 250 × 4 Bio-Sil ODS-10 (Bio-Rad)
Mobile phase: MeCN:pH 4.5 50 mM phosphate buffer 30:70 (Buffer was 6.9 g KH_2PO_4 in 1 L adjusted to pH 4.5 with orthophosphoric acid.)
Column temperature: 45
Flow rate: 2.5
Injection volume: 100
Detector: UV 202

CHROMATOGRAM

Retention time: 6.9

OTHER SUBSTANCES

Extracted: alprazolam, imipramine, nortriptyline, triazolam

Noninterfering: N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine
Interfering: protriptyline

KEY WORDS
plasma; serum

REFERENCE
McCormick,S.R.; Nielsen,J.; Jatlow,P. Quantification of alprazolam in serum or plasma by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1652–1655.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: N-desmethylclomipramine hydrochloride (10)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, nortriptyline, imipramine, 2-hydroxyimipramine, 2-hydroxydesipramine, metabolites
Also analyzed: doxepin, desmethyldoxepin, desmethylclomipramine, clomipramine, maprotiline, protriptyline
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

KEY WORDS
plasma

REFERENCE
Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 200 μL 1 μg/mL clomipramine in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2
Internal standard: clomipramine (13)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: imipramine, trimipramine
Noninterfering: monodesmethyltrimipramine, flunitrazepam, levomepromazine, alimemazine, alprazolam, amineptine, amitriptyline, caffeine, carbamazepine, citalopram, clobazam, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine
Interfering: norclobazam, triazolam

KEY WORDS
plasma

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 ng doxepin + 100 µL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 µL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 µL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 µBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 14.2
Internal standard: doxepin (12.2)

OTHER SUBSTANCES
Extracted: cocaine, dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS
desipramine is also IS; serum

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μL Serum + 50 μL 1 μg/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200 μL 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 14.9
Internal standard: N-propionylprocainamide (6)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, doxepin, imipramine, nortriptyline, protriptyline, trimipramine
Simultaneous: atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil
Interfering: desmethylmaprotiline

KEY WORDS

serum; SPE

REFERENCE

Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20

Flow rate: 1.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 7
Internal standard: minaprine (5.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: imipramine, clomipramine
Also analyzed: diltiazem, nortriptyline, amitriptyline, haloperidol, propafenone, amiodarone, verapamil

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 13
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, clomipramine, doxepin, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE
Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 150 μL 5 M NaOH, vortex, add 1 mL ethylene glycol, vortex, add 10 mL hexane, shake on a rotary shaker at 30 rpm for 30 min, centrifuge at

1000 g at 4°. Remove the organic layer and add it to 300 μL 100 mM HCl, shake at high speed for 20 min, centrifuge, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 199

CHROMATOGRAM

Retention time: 5.3
Internal standard: desipramine hydrochloride

OTHER SUBSTANCES

Simultaneous: benztropine mesylate, hyoscyamine, orphenadrine, bromocriptine, biperiden
Noninterfering: amantadine, carbidopa, levodopa

KEY WORDS

plasma; desipramine is IS

REFERENCE

Selinger,K.; Lebel,G.; Hill,H.M.; Discenza,C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma, *J.Chromatogr.*, **1989**, *491*, 248–252.

SAMPLE

Matrix: blood
Sample preparation: Make up 1-2 mL plasma to 3 mL with 100 mM NaOH, vortex for 1 min, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL diethyl ether. Add the eluate to 50 μL 5 mM phosphoric acid, evaporate under a stream of air at 40°. Add 1 mL diethyl ether to the residual solution, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 5 × 6 μBondapak C18 guard-pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 41:50:9
Flow rate: 0.8
Injection volume: 20
Detector: UV 239

CHROMATOGRAM

Retention time: 14.5
Internal standard: desipramine

OTHER SUBSTANCES

Extracted: citalopram
Simultaneous: metoclopramide, oxazepam, dihydroergotamine, lorazepam, bromazepam, methotrimeprazine, cisapride, clobazam, diazepam, cyamemazine, alimemazine
Noninterfering: heptaminol, meprobamate, caffeine

KEY WORDS

plasma; desipramine is IS; SPE

REFERENCE
Rop,P.P.; Durand,A.; Viala,A.; Jorgensen,A. Simultaneous determination of citalopram, monodesmethylcitalopram and didesmethylcitalopram in plasma by high-performance liquid chromatography after column extraction, *J.Chromatogr.*, **1990**, *527*, 226–232.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 1 mg/mL 8-hydroxychloroimipramine + 500 μL 0.6 M pH 10.4 carbonate buffer + 5 mL ethyl acetate:heptane 20:80, shake for 2.5 min, centrifuge at 3000 g for 10 min. Remove organic layer and add it to 125 μL pH 2.4 25 mM KH_2PO_4, shake for 2.5 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and put it in a rotary evaporator for 25 min to remove traces of organic solvent. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 Mm KH_2PO_4 + 5 mM tetramethylammonium chloride adjusted to pH 2.4 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, detector 1 +0.2 V, detector 2, +0.68 V, guard cell 0.70 V, gain 12 × 10, response time 0.4 s; also UV 215

CHROMATOGRAM
Retention time: 8.33
Internal standard: 8-hydroxychloroimipramine (6.35)
Limit of detection: 0.5 ng/mL (electrochemical)
Limit of quantitation: 15 ng/mL (UV)

OTHER SUBSTANCES
Simultaneous: 2-hydroxydesipramine, 2-hydroxyimipramine, imipramine, chlorodesipramine, chloroimipramine, mesoridazine
Noninterfering: doxepin, nordoxepin, amitriptyline, fluoxetine, norfluoxetine, triazolam, alprazolam

KEY WORDS
plasma

REFERENCE
Foglia,J.P.; Sorisio,D.; Perel,J.M. Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection, *J.Chromatogr.*, **1991**, *572*, 247–258.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 3 μL 20 ng/mL clobazam + 1 mL saturated sodium borate (adjusted to pH 11 with 6 M NaOH) + 5 mL n-hexane, mix 2 min, centrifuge at 3000 g for 10 min. Remove organic phase and evaporate to dryness under a stream of helium at 30°. Reconstitute in 20 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long Pelliguard LC-8 40 μm (Supelco)
Column: 150 × 4.6 C8 5 μm (Supelco)
Mobile phase: MeCN:buffer 50:50 (Buffer was 1.2 mL butylamine in 1 L 10 mM NaH_2PO_4, pH adjusted to 3 with phosphoric acid.)
Flow rate: 1
Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.282
Internal standard: clobazam (k' 1.344)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: nortriptyline, imipramine, amitriptyline, clomipramine
Simultaneous: nitrazepam, lorazepam, clonazepam, triazolam, flunitrazepam, alprazolam, diazepam, haloperidol, maprotiline

KEY WORDS
serum

REFERENCE
Segatti,M.P.; Nisi,G.; Grossi,F.; Mangiarotti,M.; Lucarelli,C. Rapid and simple high-performance liquid chromatographic determination of tricyclic antidepressants for routine and emergency serum analysis, *J.Chromatogr.*, **1991**, *536*, 319–325.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 10 mM HCl + 200 µL 10% ammonium carbonate (final pH 8.7), vortex, extract with 5 mL MTBE for 20 min (Vibrax VXR2), centrifuge at 4° at 1720 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 µL 10 mM HCl. Add 2 mL MTBE, vortex for 1 min, remove the aqueous layer and dry at 68° under high vacuum, reconstitute the residue in 100 µL mobile phase, inject a 20-40 µL aliquot.

HPLC VARIABLES
Guard column: 45 × 4.6 5 µm Ultrasphere-ODS
Column: 250 × 4.6 5 µm Ultrasphere-ODS
Mobile phase: MeOH:MeCN:40 mM ammonium acetate 38:24:38 containing 0.02% triethylamine, final pH adjusted to 7.1 with glacial acetic acid
Flow rate: 1.2
Injection volume: 20-40
Detector: UV 240

CHROMATOGRAM
Retention time: 12.9
Internal standard: desipramine

OTHER SUBSTANCES
Extracted: amlodipine

KEY WORDS
plasma; rabbit; desipramine is IS

REFERENCE
Yeung,P.K.F.; Mosher,S.J.; Pollak,P.T. Liquid chromatography assay for amlodipine: chemical stability and pharmacokinetics in rabbits, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 565–571.

SAMPLE
Matrix: blood
Sample preparation: For each 1 mL plasma or serum add 10 µL 14 µg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with

mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K_2HPO_4 adjusted to pH 6.8 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM

Retention time: 15.81
Internal standard: trimipramine (6.5)
Limit of detection: 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, clomipramine, doxepin, fluvoxamine, imipramine, maprotiline, nortriptyline
Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol

KEY WORDS

plasma; serum; column-switching

REFERENCE

Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

SAMPLE

Matrix: blood
Sample preparation: Add 10 μL 20 μg/mL oxaprotiline in MeOH to 990 μL plasma or serum. Inject 100 μL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM

Retention time: 15.8
Internal standard: oxaprotiline (9.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: clozapine, fluvoxamine, metoclopramide, fluoxetine, norfluoxetine, imipramine, nortriptyline, maprotiline, doxepin, clomipramine, amitriptyline

Noninterfering: haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Wetzel,H.; Hiemke,C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography, *Clin.Chem.*, **1992**, *38*, 2082–2086.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 μg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 μL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 11.83
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, propranolol, clovoxamine, fluvoxamine, fenfluramine, amoxapine, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine
Interfering: protriptyline

KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL trimipramine in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at

1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 6.4
Internal standard: trimipramine (9.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, doxepin, fluoxetine, imipramine, maprotiline, nortriptyline
Interfering: desmethylmaprotiline, fluvoxamine, protriptyline

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KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

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CHROMATOGRAM
Retention time: 16.7
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES

Extracted: chlorpromazine, clomipramine, cyamemazine, droperidol, flunitrazepam, haloperidol, imipramine, pipamperone, risperidone, trihexyphenidyl

Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS

plasma; SPE

REFERENCE

Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE

Matrix: blood

Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 × 4.6 LC-8-DB (Supelco)

Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)

Flow rate: 2

Injection volume: 100

Detector: UV 228

CHROMATOGRAM

Retention time: 4.0

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, quinidine, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: ibuprofen, methaqualone, norverapamil, promazine, propafenone, protriptyline

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: Stabilize plasma with 2 mg/mL sodium borohydride to prevent conversion of lofepramine to desipramine. 2 mL Plasma + 200 μL 1 M pH 11 sodium carbonate + 50 μL 1.4 μg/mL clomipramine hydrochloride in MeOH + 5 mL hexane:isoamyl alcohol 99:1, shake for 5 min, centrifuge at 3000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 100 μL mobile phase, inject a 70 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long 5 μm Supelcosil LC-PCN
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:buffer 120:35:100 (Buffer was 2.07 g/L $NaH_2PO_4.H_2O$ in water.)
Flow rate: 2.5
Injection volume: 70
Detector: UV 254

CHROMATOGRAM
Retention time: 2.80
Internal standard: clomipramine hydrochloride (3.46)
Limit of quantitation: 5 nM

OTHER SUBSTANCES
Extracted: lofepramine
Simultaneous: nortriptyline, zuclopenthixol, imipramine, perphenazine, flupentixol, amitriptyline
Interfering: haloperidol

KEY WORDS
plasma

REFERENCE
Elm,T.; Hansen,E.L. Simultaneous determination of lofepramine and desipramine by a high-performance liquid chromatographic method used for therapeutic drug monitoring, *J.Chromatogr.B*, **1995**, *665*, 355–361.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 251

CHROMATOGRAM

Retention time: 8.58
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 25 μL 5 μg/mL clomipramine in MeOH, vortex for 30 s, add 100 μL 5 M NaOH, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb MV octadecyl
Mobile phase: MeCN:10 mM triethylamine 60:40, pH adjusted to 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 5.0
Internal standard: clomipramine (8.3)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: imipramine

KEY WORDS

mouse; serum; pharmacokinetics

REFERENCE

Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Dewey,M.J. Rapid microsample analysis of imipramine and desipramine by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1995**, *668*, 338–342.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 13.3
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Homogenize brain tissue in 4 mL 50 mM pH 7.4 Tris-HCl. 500 μL Serum or 200 μL CSF or 1 mL homogenate + 100 μL 2 M NaOH + IS + 5 mL hexane: dichloromethane 60:40, shake for 15 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher RP select B
Mobile phase: MeCN:0.1% pH 4 diethylamine in water 40:60
Flow rate: 1.5
Injection volume: 30
Detector: UV 254

CHROMATOGRAM
Retention time: 8.4
Internal standard: desmethylclomipramine chlorhydrate (6.85)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, imipramine

KEY WORDS
rat; serum; brain; pharmacokinetics

REFERENCE
Besret,L.; Debruyne,D.; Rioux,P.; Bonvalot,T.; Moulin,M.; Zarifian,E.; Baron,J.-C. A comprehensive investigation of plasma and brain regional pharmacokinetics of imipramine and its metabolites during and after chronic administration in the rat, *J.Pharm.Sci.*, **1996**, *85*, 291–295.

SAMPLE
Matrix: blood, gastric contents, tissue, urine
Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH: water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES
Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 230, 254

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, pindolol, zolpidem

Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, tissue

Sample preparation: Serum. 1 mL Serum + 800 ng nortriptyline + 4 mL MeOH + 5 mL 2.5% perchloric acid, shake vigorously, centrifuge at 11000 g for 15 min. Add supernatant to 1 mL 4 M KOH, centrifuge. Add supernatant (9 mL) to 10 mL diethyl ether:ethyl acetate 85:15, shake for 15 min. Remove 8 mL of organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase buffer:MeOH 9:1, inject 100 μL aliquot. Tissue. 2 g Brain tissue + 10 mL 2.5% perchloric acid + 8 mL MeOH + 1.6 μg nortriptyline, homogenize, centrifuge at 11000 g for 15 min. Add supernatant to 4 mL 4 M KOH, centrifuge. Add supernatant to 20 mL diethyl ether:ethyl acetate 3:1, shake for 15 min. Remove 8 mL of organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase buffer:MeOH 9:1, inject 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Cosmosil 5C18

Mobile phase: MeOH:THF:buffer 45:17:88 (Buffer was 1% triethylamine adjusted to pH 3.0 with phosphoric acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 12.3

Internal standard: nortriptyline (14.2)

Limit of detection: 10 ng/g (tissue)

OTHER SUBSTANCES

Simultaneous: imipramine

KEY WORDS
serum; rat

REFERENCE
Sugita,S.; Kobayashi,A.; Suzuki,S.; Yoshida,T.; Nakazawa,K. High-performance liquid chromatographic determination of imipramine and its metabolites in rat brain, *J.Chromatogr.*, **1987**, *421*, 412–417.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 1 mL 1 M NaOH + 250 mg NaCl + 6 mL dichloromethane, shake for 15 min, centrifuge at 1500 g for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 100-150 μL aliquot. Tissue. Homogenize brain tissue gently (Ultra-Turrax) with two volumes 0.9% saline. 1 mL Homogenate + 1 mL 1 M NaOH + 250 mg NaCl + 6 mL dichloromethane, shake for 15 min, centrifuge at 1500 g for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 100-150 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm Spherisorb ODS C18
Mobile phase: MeCN:25 mM KH_2PO_4 50:50
Flow rate: 1.5
Injection volume: 100-150
Detector: UV 240

CHROMATOGRAM
Retention time: 12.2
Internal standard: desipramine

OTHER SUBSTANCES
Extracted: citalopram

KEY WORDS
plasma; desipramine is IS; rat; brain

REFERENCE
Wang,N.-S.; Lemmer,B. Determination of citalopram in plasma and brain tissue of the rat by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *488*, 492–497.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5

Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 5.88
Internal standard: cianopramine (8.93)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: protriptyline

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5.6 (A), 7.8 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum; urine) (A), 500 ng/mL (tissue), (A)

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, dothiepin, doxepin, imipramine, melitracen, mianserin, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine
Interfering: amoxapine, maprotiline

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 20 μL 10 μg/mL pericyazine in MeOH, mix, add 0.2 mL 1 M sodium carbonate buffer to adjust pH to 9.6. Add 10 mL distilled diethyl ether, shake on an automatic shaker for 10 min, centrifuge at 1000 g for 10 min in a refrigerated centrifuge. Remove the upper organic layer and add 100μL 100 mM orthophosphoric acid. Shake for 10 min and centrifuge at 1000 g for 10 min. Discard the top layer and inject a 50 μL aliquot of the acid layer.

HPLC VARIABLES
Guard column: 40 × 4.6 10 μm RP-18
Column: 300 × 3.9 Bondclone 10 C18 (Phenomenex)
Mobile phase: MeCN:100 mM K_2HPO_4, adjust pH to 6.0 with orthophosphoric acid
Flow rate: 2
Injection volume: 50
Detector: E, EDT Chromajet, oxidation cell +1.00 V

CHROMATOGRAM
Retention time: 8.7
Internal standard: pericyazine (6.2)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, imipramine

KEY WORDS
plasma; pharmacokinetics; silanize glassware

REFERENCE
Chen,A.G.; Wing,Y.K.; Chiu,H.; Lee,S.; Chen,C.N.; Chan,K. Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 153–158.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 1 mL 600 mM pH 11.3 K_2CO_3 + 100 μL 5 (plasma) or 20 (urine) μM 2-hydroxydesmethylclomipramine in EtOH + 5 mL heptane: MTBE 1:1 + 5% n-butanol, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Remove organic layer and add it to 1 mL 20 mM HCl, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Discard organic layer. Thaw out aqueous layer and make alkaline (pH 11) by adding 500 μL 600 mM pH 11.3 K_2CO_3. Add 3 mL heptane:MTBE 1:1 + 5% n-butanol, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Remove organic layer and evaporate it to dryness at 50° under a stream of nitrogen. Dissolve residue in 100 μL mobile phase, vortex for 5 s, centrifuge at 1400 g for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4 7 μm 120 Å Nucleosil
Column: 250 × 4 5 μm 100 Å Nucleosil RP-phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 14.05 g sodium perchlorate and 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 14.32
Internal standard: 2-hydroxydesmethylclomipramine (10.02)
Limit of detection: 10 nM (urine), 5 nM (plasma)

OTHER SUBSTANCES

Simultaneous: imipramine, metabolites

KEY WORDS

plasma

REFERENCE

Nielsen,K.K.; Brsen,K. High-performance liquid chromatography of imipramine and six metabolites in human plasma and urine, *J.Chromatogr.*, **1993**, *612*, 87–94.

SAMPLE

Matrix: blood, urine
Sample preparation: 500 μL Urine + 470 μL 3% L-(+)-ascorbic acid in 200 mM KH_2PO_4 + 30 μL β-glucuronidase/arylsulfatase (Boehringer Mannheim), vortex for 2 s, heat at 37° for 16 h to deconjugate, add 50 μL 2 M NaOH. 1 mL Plasma, urine, or deconjugated urine + 1 mL 600 mM pH 11.3 potassium carbonate + 100 μL EtOH + 5 mL heptane:MTBE:n-butanol 47.5:47.5:5, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze at -50°. Remove the organic layer and add it to 1 mL 20 mM HCl, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze. Discard the organic layer and add 500 μL 600 mM pH 11.3 potassium carbonate to the thawed aqueous layer. Add 3 mL heptane:MTBE:n-butanol 47.5:47.5:5 to the aqueous layer, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeOH, vortex for 5 s, centrifuge at 1400 g for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4 7 μm 120 Å Nucleosil
Column: 250 × 4 5 μm 100 Å Nucleosil RP-phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 14.05 g sodium perchlorate + 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 14.19
Internal standard: desipramine

OTHER SUBSTANCES

Extracted: clomipramine

KEY WORDS
plasma; desipramine is IS

REFERENCE
Nielsen,K.K.; Brosen,K. High-performance liquid chromatography of clomipramine and metabolites in human plasma and urine, *Ther.Drug Monit.*, **1993**, *15*, 122–128.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Serum or urine + 25 μL 5 μg/mL clomipramine in MeOH + 100 μL 5 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 5000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 10 × 4.6 5 μm Microsorb MV C18
Mobile phase: MeCN:10 mM triethylamine in water 60:40, pH adjusted to 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Internal standard: clomipramine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: imipramine

KEY WORDS
mouse; serum; pharmacokinetics

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Baumann,H.; Dewey,M.J. Altered disposition and antidepressant activity of imipramine in transgenic mice with elevated α-1-acid glycoprotein, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 918–922.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.87

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Extract ground tablets containing 1 mg with 10 mL 1% HCl, shake for 30 min, centrifuge at 2000 rpm for 5 min. Remove a 5 mL aliquot of the supernatant and add it to 10 mL 1.25 mg/mL norephedrine hydrochloride in MeOH, make up to 25 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeOH:MeCN:25 mM pH 4.5 acetate buffer 30:40:30
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.2
Internal standard: norephedrine (2.38)

OTHER SUBSTANCES
Interfering: fluphenazine, promazine

KEY WORDS
tablets

REFERENCE
Beaulieu,N.; Gagné,C.; Lovering,E.G. Liquid chromatographic determination of identity, content, and content uniformity of desipramine, fluphenazine, and promazine, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 178–179.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18

Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, dothiepin, doxepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a C18 SepPak SPE cartridge with 5 mL MeOH and 5 mL buffer. 1 mL Microsomal incubation + 500 μL buffer, mix, add a 500 μL aliquot to the SPE cartridge, wash with 4 mL MeOH:buffer containing 0.01% ascorbic acid 5:95, elute with 5 mL MeOH, evaporate eluate to dryness, reconstitute in mobile phase, inject an aliquot. (Buffer was 100 mM pH 3.0 potassium acetate buffer containing 5 mM n-heptanesulfonic acid.)

HPLC VARIABLES
Guard column: used but not specified
Column: 100 × 8 4 μm Nova-Pak C18
Mobile phase: Gradient. MeOH:MeCN:buffer 20:35:45 for 20 min, then 30:50:20 for 25 min, then 35:60:5
Flow rate: 0.4
Injection volume: 50-300
Detector: UV 254

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Extracted: metabolites, imipramine, lofepramine

KEY WORDS
rat; human; liver; SPE

REFERENCE
Strandgården,K.; Gunnarsson,P.O. Metabolism of lofepramine and imipramine in liver microsomes from rat and man, *Xenobiotica*, **1994**, *24*, 703–711.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation + 50 μL MeCN, cool on ice, add 2.5 μg hydroxyclomipramine, centrifuge at 14000 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 40:60 containing 1 mL/L acetic acid
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Internal standard: hydroxyclomipramine (11)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; human

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Cotreau-Bibbo,M.M.; Duan,S.X.; Harmatz,J.S.; Shader,R.I. Inhibition of desipramine hydroxylation in vitro by serotonin-reuptake-inhibitor antidepressants and by quinidine and ketoconazole: A model system to predict drug interactions in vivo, *J.Pharmacol.Exp.Ther.*, **1994**, *268*, 1278–1283.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 500 μL 2 M pH 12 sodium carbonate + 5 mL ether, vortex, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 1 mL 100 mM HCl, vortex, centrifuge. Remove the aqueous layer and add it to 100 μL 2 M pH 12 sodium carbonate and 1 mL ether, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7 K_2HPO_4 40:35:25
Flow rate: 1.4
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 12.9
Limit of detection: 50 pM

OTHER SUBSTANCES
Extracted: imipramine

KEY WORDS
rat; liver; brain

REFERENCE
Sequeira,D.J.; Strobel,H.W. High-performance liquid chromatographic method for the analysis of imipramine metabolism in vitro by liver and brain microsomes, *J.Chromatogr.B*, **1995**, *673*, 251–258.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.16

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 18.3

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)

Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: amitriptyline, desmethyldoxepin, doxepin, imipramine, loxapine, maprotiline, nortriptyline, trazodone

REFERENCE
Supelco Catalog, **1993**, p. 440.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 7.3
Limit of quantitation: < 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: doxepin, protriptyline, cyclobenzaprine, maprotiline
Also analyzed: amitriptyline, amoxapine, carbamazepine, imipramine, nortriptyline

KEY WORDS
UV spectra given

REFERENCE
Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 13.5

OTHER SUBSTANCES
Simultaneous: desmethyldoxepin, doxepin, nortriptyline, imipramine, amitriptyline

Also analyzed: amphetamine, chlordiazepoxide, chlorpromazine, desalkylflurazepam, diazepam, diethylpropion, ephedrine, fenfluramine, flurazepam, mesoridazine, methamphetamine, norchlordiazepoxide, nordiazepam, oxazepam, phentermine, phenylpropanolamine, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 4.77

OTHER SUBSTANCES

Simultaneous: benactyzine, buclizine, hydroxyzine, perphenazine, thioridazine, amitriptyline, imipramine, nortriptyline, protriptyline

KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide,

cymarin, danazol, danthron, dapsone, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 13.00 (A), 6.26 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.28

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.80

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 3.1

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine

Interfering: pentazocine, methamphetamine, nortriptyline, diphenhydramine

KEY WORDS

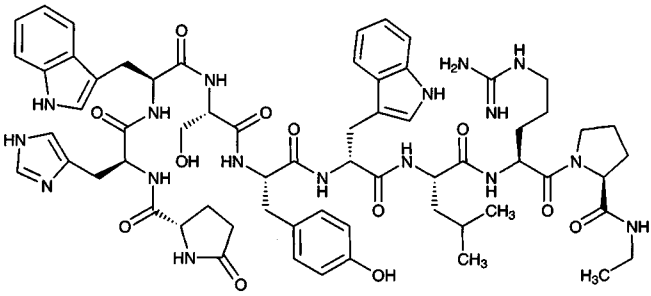
column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Deslorelin

Molecular formula: $C_{64}H_{83}N_{17}O_{12}$
Molecular weight: 1282.47
CAS Registry No.: 57773-65-6
Merck Index: 2968



SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 3 Spherisorb S5ODS-2
Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$. B was MeCN. A:B from 82:18 to 64:36 over 25 min, maintain at 64:36 for 2.5 min, return to initial conditions over 1 min, re-equilibrate for 6.5 min. or Isocratic MeCN:0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$ 24:76
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 23.5 (gradient), 26 (isocratic)

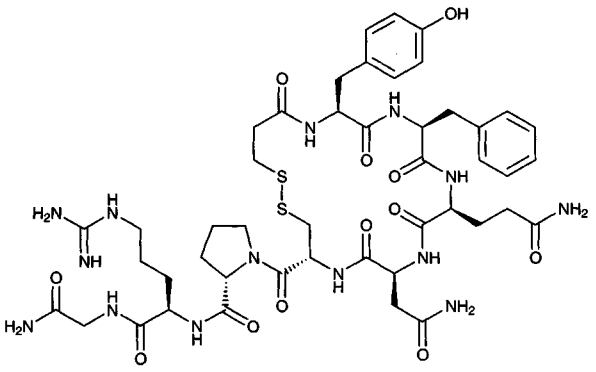
OTHER SUBSTANCES
Simultaneous: buserelin, gonadorelin, goserelin, leuprolide, nafarelin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Corran,P.H.; Sutcliffe,N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography, *J.Chromatogr.*, **1993**, *636*, 87–94.

Desmopressin

Molecular formula: $C_{46}H_{64}N_{14}O_{12}S_2$
Molecular weight: 1069.23
CAS Registry No.: 16679-58-6, 62357-86-2 (acetate trihydrate), 62288-83-9 (acetate)
Merck Index: 2969



SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 mM pH 7.40 phosphate buffer + 500 μL 2% zinc sulfate in MeOH:water 50:50, mix, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 3 5 μm Spherisorb ODS-2
Mobile phase: MeCN:0.1% phosphoric acid 20:80 containing 1 mM triethylamine
Flow rate: 1
Injection volume: 20
Detector: UV 215

KEY WORDS

plasma

REFERENCE

Kahns,A.H.; Buur,A.; Bundgaard,H. Prodrugs of peptides. 18. Synthesis and evaluation of various esters of desmopressin (dDAVP), *Pharm.Res.*, **1993**, *10*, 68–74.

SAMPLE

Matrix: solutions

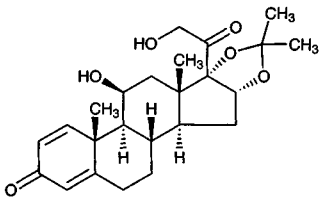
HPLC VARIABLES

Column: 125 × 4.0 4 μm Superspher 100 RP
Mobile phase: MeCN:MeOH:70 mM pH 5.2 phosphate buffer 13:12:75, containing 800 μ g/mL sodium n-butanesulfonate
Injection volume: 50
Detector: UV 220

REFERENCE

Nakakura,M.; Kato,Y.; Ito,K. Safe and efficient transdermal delivery of desmopressin acetate by iontophoresis in rats, *Biol.Pharm.Bull.*, **1998**, *21*, 268–271.

Desonide



Molecular formula: $C_{24}H_{32}O_6$
Molecular weight: 416.51
CAS Registry No.: 638-94-8
Merck Index: 2973
Lednicer No.: 2 179

SAMPLE

Matrix: formulations

Sample preparation: Condition a 100 mg Bond Elut silica SPE cartridge with one column volume hexane:chloroform 80:20. Weigh out 250-300 mg cream or ointment, add 1 mL 250 μg/mL butyl hydroxybenzoate in chloroform, make up to 25 mL with hexane-chloroform 80:20, shake gently (whirlmixer), add 500 μL of the solution to the SPE cartridge, wash with three column volumes of hexane, add 500 μL MeOH, after 1-2 min apply vacuum to elute, repeat process, combine eluates, shake gently, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Spheri-5 MPLC C18
Column: 100 × 4.6 Spheri-5 MPLC C18
Mobile phase: MeOH:water 60:40
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5
Internal standard: butyl hydroxybenzoate (7)

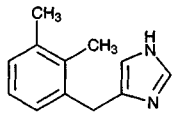
KEY WORDS

cream; ointment; SPE

REFERENCE

Nguyen,T.T.; Kringstad,R.; Rasmussen,K.E. Use of extraction columns for the isolation of desonide and parabens from creams and ointments for high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1986**, *366*, 445–450.

Detomidine



Molecular formula: $C_{12}H_{14}N_2$
Molecular weight: 186.26
CAS Registry No.: 76631-46-4, 90038-01-0 (HCl)
Merck Index: 2981
Lednicer No.: 5 71

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 5 mL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 75:25. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hitachi gel 3056
Mobile phase: MeOH:100 mM ammonium acetate 65:35
Flow rate: 1
Injection volume: 50
Detector: MS, Hitachi M-1000, APCI interface, drift voltage 21 V, nebulizer 260°, vaporizer 399°, multiplier voltage 1500 VF, m/z 187

CHROMATOGRAM

Retention time: 6.5
Internal standard: detomidine

OTHER SUBSTANCES

Extracted: atipamazole, medetomidine, midazolam

KEY WORDS

pig; plasma; SPE; detomidine is IS

REFERENCE

Kanazawa,H.; Nishimura,R.; Sasaki,N.; Takeuchi,A.; Takai,N.; Nagata,Y.; Matsushima,Y. Determination of medetomidine, atipamazole and midazolam by liquid chromatography-mass spectrometry, *Biomed.Chromatogr.*, **1995**, *9*, 188–191.

SAMPLE

Matrix: cell incubations, urine
Sample preparation: Filter urine or cell incubations and inject an aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate from 6:94 to 50:50 over 33 min
Flow rate: 1
Injection volume: 50
Detector: UV 215 or radioactivity

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES

Extracted: metabolites

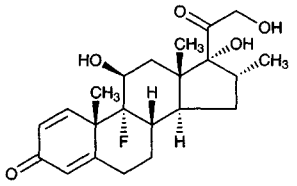
KEY WORDS

rat

REFERENCE

Salonen,J.S.; Suolinna,E.-M. Metabolism of detomidine in the rat. I. Comparison of ^{3}H-labelled metabolites formed in vitro and in vivo, *Eur.J.Drug Metab.Pharmacokinet.*, **1988**, *13*, 53–58.

Dexamethasone



Molecular formula: $C_{22}H_{29}FO_5$

Molecular weight: 392.47

CAS Registry No.: 50-02-2, 1177-87-3 (acetate), 55812-90-3 (acetate monohydrate), 2392-39-4 (sodium phosphate), 83880-70-0 (acefurate), 55541-30-5 (dipropionate), 312-93-6 (21-dihydrogen phosphate)

Merck Index: 2986

Lednicer No.: 1 199

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 10 μL 150 ng/mL beclomethasone in MeOH + 300 μL water + 6 mL diethyl ether, shake for 5 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen. Dissolve in 100 μL MeOH. Add 20 μL copper acetate solution and let stand at room temperature for 1 h. Add 80 μL diaminophthalhydrazide solution, heat at 80° for 110 min, cool, inject a 20 μL aliquot. (Prepare copper acetate solution by dissolving 700 mg copper(II) acetate in 10 mL water, dilute to 100 mL with MeOH, discard after 1 month. Diaminophthalhydrazide solution was 7.5 mM 4,5-diaminophthalhydrazide in 3.5 M hydrochloric acid containing 625 mM β-mercaptoethanol, discard after 5 h. Prepare 4,5-diaminophthalhydrazide dihydrochloride as follows. Reflux 316 g 4-nitrophthalic acid and 50 mL concentrated sulfuric acid in 500 mL MeOH for 10 h, recrystallize the product (dimethyl 4-nitrophthalate) from MeOH (mp 64-65E). Hydrogenate 47.8 g dimethyl 4-nitrophthalate in 300 mL MeOH over 13 g 5% platinum on carbon at an initial hydrogen pressure of 50 psi. When the calculated amount of hydrogen has been absorbed remove the catalyst and evaporate to dryness under reduced pressure, recrystallize the residue from aqueous MeOH to give dimethyl 4-aminophthalate (mp 83-84E). Stir 146.3 g dimethyl 4-aminophthalate in 1.4 L acetic anhydride at 60-70E for 2 h then leave overnight, precipitate product with MeOH. Dry the product and rinse it with sodium carbonate solution, re-dry, recrystallize from benzene/MeOH (Caution! Benzene is a carcinogen!) to give dimethyl 4-acetamidophthalate (mp 138-140E). Add 100.4 g to 600 mL fuming (90%) nitric acid at 0-5E over 30 min, stir at 5-10E for 2.5 h, mix the reaction mixture with 800 mL cold dichloromethane, shake with crushed ice. Remove the organic layer and extract the aqueous layer with 200 mL cold dichloromethane. Combine the organic layers and wash them with ice water, cold sodium bicarbonate solution, and cold water. D ry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure and, recrystallize repeatedly from MeOH to give dimethyl 4-acetamido-5-nitrophthalate (mp 123-124.5E). Hydrolyze dimethyl 4-acetamido-5-nitrophthalate to dimethyl 4-amino-5-nitrophthalate. Hydrogenate 20.3 g dimethyl 4-amino-5-nitrophthalate in 250 mL MeOH over 1 g 5% platinum on carbon at an initial hydrogen pressure of 50 psi, remove the catalyst, evaporate to dryness under reduced pressure at 25E, recrystallize from chloroform/dichloromethane to give dimethyl 4,5-diaminophthalate (mp 111.5-113E). Add 1.1 g dimethyl 4,5-diaminophthalate to 3 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) and 3 mL triethylamine in 20 mL MeOH, concentrate the resulting solution, triturate with benzene/MeOH, recrystallize from N,N'-dimethylacetamide/acetic acid to give 4,5-diaminophthalhydrazide (6,7-diamino-2,3-dihydrophthalazine-1,4-dione) (mp 407E) (J. Heterocycl. Chem. 1973, 10, 891), mix 4,5-diaminophthalhydrazide with a small amount of concentrated HCl, recrystallize from EtOH to give 4,5-diaminophthalhydrazide dihydrochloride.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSKgel ODS-120T (Tosoh, Japan)

Mobile phase: MeCN:tetrahydrofuran:100 mM pH 7.0 phosphate buffer 24:3:73

Flow rate: 1.0

Injection volume: 20

Detector: Chemiluminiscence following post-column reaction. The column effluent mixed with 20 mM hydrogen peroxide in water pumped at 1.0 mL/min and then with 30 mM potassium hexacyanoferrate(III) in 3.0 M NaOH pumped at 2.0 mL/min and flowed to the detector.

CHROMATOGRAM
Retention time: 32
Internal standard: beclomethasone (52)
Limit of detection: 200 pg/mL

OTHER SUBSTANCES
Simultaneous: aldosterone, corticosterone, cortisone, 11-deoxycortisol, hydrocortisone, 18-hydroxycorticosterone, 18-hydroxydeoxycorticosterone, prednisolone, prednisone
Noninterfering: androstendione, cholesterol, estrone, estradiol, estriol, pregnenolone, progesterone
Interfering: betamethasone.

KEY WORDS
plasma; derivatization

REFERENCE
Ishida,J.; Sonezaki,S.; Yamaguchi,M.; Yoshitake,T., Determination of dexamethasone in plasma by high-performance liquid chromatography with chemiluminiscence detection, *Anal.Sci.*, **1993**, *9*, 319–322.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL EtOH:water 10:90 + 100 μL 250 mM NaOH + 7 mL ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100 μL dichloromethane:EtOH:water 95:4:1, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Partisil silica
Mobile phase: Dichloromethane:EtOH:water 95:4:1
Flow rate: 1.5
Injection volume: 50
Detector: UV 239

CHROMATOGRAM
Retention time: 11.5
Internal standard: dexamethasone

OTHER SUBSTANCES
Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 17-hydroxyprogesterone, 6α-methylprednisolone, prednisolone, prednisone, progesterone

KEY WORDS
plasma; normal phase; dexamethasone is IS

REFERENCE
Scott,N.R.; Chakraborty,J.; Marks,V. Determination of prednisolone, prednisone, and cortisol in human plasma by high-performance liquid chromatography, *Anal.Biochem.*, **1980**, *108*, 266–268.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 5 mL water + 1 mL 2 μg/mL equilenin in MeOH + 50 μL 0.1 M NaOH to adjust pH to 10, vortex briefly after each addition, shake with 10 mL dichloromethane for 10 min, centrifuge at 2000 g for 10 min. Wash organic layer twice with 2 mL water, centrifuge 5 min, evaporate 8 mL of organic phase to dryness at 40° under a stream of nitrogen, reconstitute residue in 150 μL mobile phase, inject 25 μL aliquot

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HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer 60:40 (Buffer was 10 mL 200 mM acetic acid + 15 mL 200 mM sodium acetate made up to 1 L, pH 4.8.)
Flow rate: 1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: equilenin (7.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: deoxycortisol, hydrocortisone, prednisone, triamcinolone, prednisolone
Interfering: betamethasone

KEY WORDS
plasma

REFERENCE
Bouquet,S.; Brisson,A.M.; Gombert,J. Dosage du cortisol et du 11-désoxycortisol plasmatiques par chromatographie liquide haute performance [Cortisol and 11-desoxycortisol determination in blood by high performance liquid chromatography], *Ann.Biol.Clin.(Paris)*, **1981**, *39*, 189–191.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 10 μg/mL prednisolone in MeOH, add 1 mL 0.1 M NaOH, add 10 mL dichloromethane, shake for 10 min, centrifuge at 8400 g at 4° for 10 min. Remove organic layer and evaporate it at 40° under a stream of nitrogen. Dissolve residue in 100 μL mobile phase and inject.

HPLC VARIABLES
Column: 100 × 8 radial compression 10 μm Radialpack B
Mobile phase: Dichloromethane:MeOH:acetic acid 96:4:0.4
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 5.0
Internal standard: prednisolone (7.5)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrocortisone, corticosterone

KEY WORDS
plasma; dog; normal phase

REFERENCE
Alvinerie,M.; Toutain,P.L. Simultaneous determination of corticosterone, hydrocortisone, and dexamethasone in dog plasma using high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 816–818.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 10 mL ether:dichloromethane 60:40, shake for 10 min, centrifuge at 300 g for 5 min. Remove the organic layer and add it to 1 mL 100 mM HCl, shake for 5 min, centrifuge at 300 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak/Corasil (Waters)
Column: 300 × 3.9 10 μm μPorasil (Waters)
Mobile phase: Dichloromethane:glacial acetic acid 99:1 (Prepare dichloromethane as follows. Stir 500 mL dichloromethane, 30 mL EtOH, and 30 mL water for 1 h, use the lower organic layer.)
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: dexamethasone

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisolone, prednisone

KEY WORDS
plasma; normal phase; dexamethasone is IS

REFERENCE
Hartley,R.; Brocklebank,J.T. Determination of prednisolone in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *232*, 406–412.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 80 μL 3.125 μg/mL dexamethasone in MeOH, mix, add 15 mL dichloromethane, shake for 20 min, centrifuge. Remove organic phase and wash it with 1 mL 100 mM NaOH then with 1 mL water. Remove organic phase and dry it with 1 g anhydrous sodium sulfate. Evaporate to dryness at 45° under a stream of nitrogen, reconstitute in 200 μL mobile phase, inject.

HPLC VARIABLES
Guard column: 70 × 6 37-53 μm Whatman HC-Pellocil
Column: 250 × 4.6 5-6 μm Zorbax SIL
Mobile phase: Hexane:dichloromethane:ethanol:acetic acid 26:69:3.4:1
Flow rate: 2
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: dexamethasone
Limit of detection: 2 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: methylprednisolone, methylprednisone, hydrocortisone, fluocinonide, cortisone, corticosterone, prednisone, beclomethasone, betamethasone, prednisolone

KEY WORDS
plasma; normal phase; dexamethasone is IS

REFERENCE
Ebling,W.F.; Szefler,S.J.; Jusko,W.J. Analysis of cortisol, methylprednisolone, and methylprednisolone hemisuccinate. Absence of effects of troleandomycin on ester hydrolysis, *J.Chromatogr.*, **1984**, *305*, 271–280.

SAMPLE
Matrix: blood
Sample preparation: Prepare a Bond-Elut C18 SPE column by washing with 2 mL MeCN, 2 mL acetone:water 2:98, and 4 mL water. Do not allow column to run dry. Add 2 mL plasma to SPE cartridge, allow to sit for 15 min, wash twice with 2 mL water, wash twice with 2 mL acetone:water 2:98, pull a vacuum on the column for 15 min, elute with 1 mL MeCN under vacuum. Evaporate the eluate to dryness under a stream of nitrogen at 40°, dissolve the residue in 150 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb Si-60
Mobile phase: Dichloromethane:water-saturated dichloromethane:THF:MeOH:glacial acetic acid 664.5:300:10:25:0.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 23.5
Internal standard: dexamethasone

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisone, cortisone, prednisolone, prednisolone acetate

KEY WORDS
plasma; normal phase; pig; dexamethasone is IS; SPE

REFERENCE
Prasad,V.K.; Ho,B.; Haneke,C. Simultaneous determination of prednisolone acetate, prednisolone, prednisone, cortisone and hydrocortisone in swine plasma using solid-phase and liquid-liquid extraction techniques, *J.Chromatogr.*, **1986**, *378*, 305–316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 5 mL dichloromethane:diethyl ether 50:50, vortex for 15 s, repeat extraction, combine organic layers and wash them with 4 mL 100 mM NaOH, centrifuge. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, dissolve the residue in 150 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb Si-60

Mobile phase: Dichloromethane:water-saturated dichloromethane:THF:MeOH:glacial acetic acid 664.5:300:10:25:0.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 23.5
Internal standard: dexamethasone

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisone, cortisone, prednisolone, prednisolone acetate

KEY WORDS
plasma; normal phase; pig; dexamethasone is IS

REFERENCE
Prasad,V.K.; Ho,B.; Haneke,C. Simultaneous determination of prednisolone acetate, prednisolone, prednisone, cortisone and hydrocortisone in swine plasma using solid-phase and liquid-liquid extraction techniques, *J.Chromatogr.*, **1986**, *378*, 305–316.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL serum to a Sep Pak C18 SPE cartridge, wash with 4 mL water, elute with 4 mL MeOH, evaporate to dryness under vacuum, reconstitute in 50 μL MeCN:water 30:70, inject whole sample.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 30:70
Flow rate: 1
Injection volume: 50
Detector: enzyme immunoassay of fractions

CHROMATOGRAM
Retention time: 17
Limit of detection: 0.3 pg

OTHER SUBSTANCES
Extracted: betamethasone, flumethasone, triamcinolone
Noninterfering: endogenous steroids

KEY WORDS
serum; SPE; horse

REFERENCE
Friedrich,A.; Schulz,R.; Meyer,H.H. Use of enzyme immunoassay and reverse-phase high-performance liquid chromatography to detect and confirm identity of dexamethasone in equine blood, *Am.J.Vet.Res.*, **1992**, *53*, 2213–2220.

SAMPLE
Matrix: blood
Sample preparation: Condition a Tef Elutor C18 cartridge with two 3 mL portions of MeOH then two 3 mL portions of water. 1 mL Plasma + 50 μL 400 ng/mL flumethasone in 5:95 MeOH:water, heat at 50° for 10 min, add to cartridge, wash with 2 mL water, 1 mL MeOH:water 10:90, 4 mL acetone:water 20:80, apply suction to cartridge for 10 min

to air dry. Elute with 1 mL MeOH, evaporate eluent at 45° under nitrogen, reconstitute with 50 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm C18 Hypersil
Mobile phase: MeCN:THF:water 8:10:82, containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 8.34
Internal standard: flumethasone (11.50)
Limit of detection: 300 pg/mL

OTHER SUBSTANCES

Simultaneous: prednisone, hydrocortisone, adrenosterone, prednisolone, estriol, corticosterone, methylprednisolone, cortisone, hydroxyprogesterone, testosterone, deoxycorticosterone, fluorometholone, spironolactone, equilenin, estrone, estradiol, progesterone, diphenhydramine, propranolol, aspirin, theophylline, imipramine, desipramine, indomethacin, amitriptyline, nortriptyline, nordiazepam, diazepam, chlordiazepoxide, tripelennamine, carbamazepine, probenecid, phenobarbital
Noninterfering: caffeine, nicotine, cotinine, chlorothiazide, acetazolamide, phenytoin, pheniramine, cephalothin, primidone, acebutolol, hydrochlorothiazide, quinine, acetophenetidin, furosemide, aldosterone, triamcinolone, ephedrine, allopurinol, phenylephrine

KEY WORDS

plasma; SPE

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography, *Clin.Chem.*, **1992**, *38*, 346–352.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL IS in water, extract twice by shaking for 1 min with 1.2 mL dichloromethane, evaporate organic layer below 40° under reduced pressure, dissolve residue in 100 μL MeCN. Add 10 μL reagent 1, add 10 μL reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μL water, add 200 μL MeOH:water 1:1, add to Sep-Pak C18 cartridge, wash vial with 2 mL MeOH:water 1:1 and add washings to cartridge, wash cartridge with 40 mL MeOH:water 1:1, elute with 5 mL MeOH. Concentrate eluent to 500 μL by evaporation at 40° under reduced pressure, inject 20 μL aliquot. (Reagent 1 was 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, add 700 mg 4-piperidinopyridine, dilute to 10 mL with MeCN. Reagent 2 was 700 mg 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in 10 mL MeCN. Prepare 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as follows. Add 13 g 4-carboxybenzaldehyde (terephthalaldehydic acid) in 400 mL EtOH dropwise to 4,5-dimethyl-1,2-phenylenediamine in 400 mL EtOH in an ice bath, after 1 h reflux for 8 h, cool to room temperature, collect the precipitate, recrystallize three times from MeOH:water 50:50 to give 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as a white amorphous product (mp >300°) (J.Chromatogr. 1991, 585, 219). 4-Piperidinopyridine is not commercially available but 4-dimethylaminopyridine or 4-pyrrolidinopyridine can be used instead although interferences are greater (J. Chromatogr. 1991, 585, 219). Alternatively 4-piperidinopyridine can be synthesized as follows. Add 200 mmoles piperidine dropwise with stirring to 15 g phosphorus pentoxide and 9.51 g 4-hydroxypyridine, heat at 250° for 7 h, cautiously pour onto 200 g ice, add 400 mL 1 M NaOH, add 200 mL ether. Remove the ether layer and extract the aqueous layer three times with 100 mL portions of ether. Combine the organic

layers and dry them over anhydrous potassium carbonate, evaporate, distil the residue, recrystallize from petroleum ether (bp 80-100°) to give 4-piperidinopyridine (bp 167-170°/11 mm Hg; mp 79-80°) (Synthesis 1978, 844). Alternatively, add 1.94 g 4-bromopyridine hydrochloride to 5 mL 50% NaOH, add 5 mL piperidine, add 2.72 g benzyltriethylammonium bromide, heat at 100° for 5 h, remove excess piperidine by distillation, add 25 mL water, extract four times with 25 mL portions of benzene. Combine the organic layers and dry them over anhydrous sodium sulfate, boil the residue with petroleum ether to give 4-piperidinopyridine (mp 80°) (Syn. Commun. 1979, 9, 251). Prepare 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate as follows. Stir 1.41 moles isopropylisocyanate in 750 mL dichloromethane at 5°, add 144 g 3-dimethylaminopropylamine (N,N-dimethyl-1,3-propanediamine) in 250 mL dichloromethane at such a rate that the temperature does not exceed 10°, add 500 mL triethylamine, add 300 g p-toluenesulfonyl chloride in 300 mL dichloromethane at such a rate that the temperature does not exceed 10°, reflux for 3 h, add 400 g anhydrous sodium carbonate, add 3.5 L ice water, stir vigorously for 30 min, remove the organic phase. Extract the aqueous phase three times with 500 mL portions of dichloromethane. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate under reduced pressure, distil the residue to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide (bp 91-92°/10 mm Hg (Ber. 1941, 74B, 1285)) (cf. Org. Syn. 1973, Coll. Vol. V, 555). Prepare pyridine perchlorate from pyridine and 20% perchloric acid, crystallize from EtOH (Ber. 1926, 59, 446). Add 18 g pyridine perchlorate in portions to 100 mmoles 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide stirred in 200 mL dichloromethane at 0°, let stand for 30 min, filter, add 200 mL anhydrous diethyl ether to the filtrate. Filter off the precipitate and recrystallize it from dichloromethane/diethyl ether to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate (mp 88-90°) (Chem. Pharm. Bull. 1985, 33, 5375).)

HPLC VARIABLES

Guard column: 50 × 4.6 7 μm Zorbax ODS
Column: 250 × 4.6 7 μm Zorbax ODS
Mobile phase: MeOH:water 75:25 containing 5 mM tetramethylammonium hydrogen sulfate
Flow rate: 0.4
Injection volume: 20
Detector: F ex 334 em 418

CHROMATOGRAM

Retention time: 33.1
Internal standard: fluocinolone acetonide (40.7)
Limit of detection: 0.6-3 pg/mL

OTHER SUBSTANCES

Simultaneous: aldosterone, corticosterone, hydrocortisone, cortisone, triamcinolone

KEY WORDS

plasma; derivatization

REFERENCE

Katayama,M.; Masuda,Y.; Taniguchi,H. Determination of corticosteroids in plasma by high-performance liquid chromatography after pre-column derivatization with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole, *J.Chromatogr.*, **1993**, *612*, 33–39.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 25:20:55
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Internal standard: dexamethasone

OTHER SUBSTANCES
Simultaneous: dibucaine

KEY WORDS
plasma; rat; dexamethasone is IS

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 1 mL 50 ng/mL beclomethasone in ethyl acetate, vortex, centrifuge at 11000-12300 g for 5 min. Evaporate the supernatant under a stream of nitrogen at 50-60°, reconstitute in 100 μL mobile phase, inject a 20-80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 45:55
Flow rate: 1
Injection volume: 20-80
Detector: UV 240

CHROMATOGRAM
Retention time: 18
Internal standard: beclomethasone (22)
Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES
Extracted: hydrocortisone
Noninterfering: albuterol, amoxicillin, ceftriaxone, erythromycin, furosemide, gentamicin, indomethacin, midazolam, morphine, nystatin, theophylline, vancomycin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Schild,P.N.; Charles,B.G. Determination of dexamethasone in plasma of premature neonates using high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 189–192.

SAMPLE
Matrix: blood
Sample preparation: 750 μL Serum + 175 μL MeOH + 2 mL ethyl acetate, shake for 10 min, centrifuge at 2500 g for 10 min. Remove 1.9 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL ethyl acetate, inject a 17 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 5 μm LiChrosorb Si 60
Column: 250 × 4 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:dichloromethane:MeOH:acetic acid 266:120:26:0.8 (Prepare by mixing an aliquot of mobile phase with an aliquot of mobile phase saturated with water.)
Flow rate: 2
Injection volume: 17
Detector: UV 242

CHROMATOGRAM

Retention time: 11.43
Internal standard: dexamethasone

OTHER SUBSTANCES

Extracted: hydrocortisone, prednisolone, prednisolone acetate

KEY WORDS

serum; normal phase; dexamethasone is IS

REFERENCE

Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of prednisolone, prednisolone acetate and hydrocortisone in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 237–246.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM

Retention time: 19.99
Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES

Extracted: betamethasone, chloroquine, corticosterone, cortisone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum or urine + 500 μL 200 mM pH 3.85 acetate buffer (serum only) + 400 μL 2.5 μM IS in mobile phase, mix, centrifuge. Add the supernatant to the SPE cartridge, wash with 3 mL acetone:water 20:80, 3 mL water, and 3 mL hexane. Elute with 3 mL diethyl ether into tubes containing 1 mL 200 mM NaOH, vortex, centrifuge. Dry the organic layer under a stream of nitrogen. Reconstitute the residue in 250 μL mobile phase, mix for 5 min. Inject a 60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherex C18 (Phenomenex USA)
Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 29.85
Internal standard: fludrocortisone (15.9)
Limit of detection: 5 nM

OTHER SUBSTANCES
Extracted: 11-deoxycortisol, hydrocortisone, methylprednisolone, prednisolone

KEY WORDS
serum; SPE

REFERENCE
McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 241.7

CHROMATOGRAM
Retention time: 13.127

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: If necessary, dilute injection 1:9 with mobile phase (for 50 mL admixtures) and 1:4 (for 100 mL admixtures), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select B
Mobile phase: MeCN:buffer 25:75 (Buffer was 20 mM KH_2PO_4 adjusted to pH 6.0 with NaOH solution.)
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: ondansetron

KEY WORDS
5% dextrose; 0.9% sodium chloride; injections; stability-indicating

REFERENCE
Evrard,B.; Ceccato,A.; Gaspard,O.; Delattre,L.; Delporte,J.-P. Stability of ondansetron hydrochloride and dexamethasone sodium phosphate in 0.9% sodium chloride injection and in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 1065–1068.

SAMPLE
Matrix: formulations
Sample preparation: One tablet + 5 mL acetone:ethanol, sonicate 5 min, centrifuge at 1400 g for 5 min, evaporate supernatant under vacuum, dissolve residue in 100 μL MeOH, inject 0.2 μL aliquots.

HPLC VARIABLES
Column: 95 × 0.5 Japan Spectroscopic SC-01 (5 μm octadecylsilyl silica in a PTFE tube)
Mobile phase: MeCN:water 28:72
Flow rate: 0.008
Injection volume: 0.2
Detector: UV 220

CHROMATOGRAM
Retention time: 14

Internal standard: dexamethasone

OTHER SUBSTANCES
Simultaneous: digoxin, digoxigenin, digoxigenin monodigitoxoside, digoxigenin bisdigitoxoside, β-methyldigoxin, dimethyldigoxin

KEY WORDS
tablets; microbore; dexamethasone is IS

REFERENCE
Fujii,Y.; Ikeda,Y.; Yamazaki,M. High-performance liquid chromatographic determination of secondary cardiac glycosides in Digitalis purpurea leaves, *J.Chromatogr.*, **1989**, *479*, 319–325.

SAMPLE
Matrix: formulations
Sample preparation: Triturate 1 tablet with a glass rod with 5 mL water, sonicate for 20 min, extract with 9 mL dichloromethane then three times with 5 mL dichloromethane, filter (paper), make up to 25 mL with dichloromethane. Remove a 500 μL aliquot, add 200 μL 1.2 mM phenacetin in dichloromethane + 100 μL 0.5 mM 4-dimethylaminopyridine + 100 μL 100 mM N-CBZ-Phe in dichloromethane + 100 μL 100 mM N,N'-dicyclohexylcarbodiimide in dichloromethane, shake mechanically at 30° for 1 h, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 75 × 3.9 4 μm Nova-Pak silica
Mobile phase: n-Hexane:dichloromethane:isopropanol 100:100:4
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 5
Internal standard: phenacetin (10)
Limit of detection: 4.2 pmol

OTHER SUBSTANCES
Simultaneous: betamethasone

KEY WORDS
tablets; normal phase; derivatization

REFERENCE
Chen,S.-H.; Wu,S.-M.; Wu,H.-L. Stereochemical analysis of betamethasone and dexamethasone by derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *595*, 203–208.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets, weigh out amount equivalent to about 500 μg betamethasone, add 10 mL water, sonicate for 15 min, extract three times with 15 mL chloroform:n-butanol 95:5. Combine extracts and filter them through 1 g anhydrous sodium sulfate moistened with chloroform:n-butanol 95:5. Collect filtrate and dilute it to 50 mL with chloroform:n-butanol 95:5. Remove a 1 mL aliquot, add 0.5 mL 40 μM cortisone in mobile phase, mix, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Guard-Pak Resolve Si (dead volume 60-75 μL)
Column: 75 × 3.9 4 μm Nova-Pak silica

Mobile phase: Dichloromethane:EtOH 34:1
Flow rate: 0.7
Injection volume: 5
Detector: UV 240

CHROMATOGRAM
Retention time: 5
Internal standard: cortisone (3)

OTHER SUBSTANCES
Simultaneous: betamethasone, prednisone, hydrocortisone, prednisolone, 6α-methylprednisolone

KEY WORDS
tablets; normal phase

REFERENCE
Liu,K.-R.; Chen,S.-H.; Wu,S.-M.; Kou,H.-S.; Wu,H.-L. High-performance liquid chromatographic determination of βmethasone and dexamethasone, *J.Chromatogr.A*, **1994**, *676*, 455–460.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water.

HPLC VARIABLES
Column: Bakerbond phenyl
Mobile phase: MeOH:buffer 26:74 (Buffer was 7.5 mL triethylamine in 1 L water adjusted to pH 5.4 with orthophosphoric acid.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7

KEY WORDS
saline; injections; stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J.; Holland,J.S. Stability of dexamethasone sodium phosphate, diphenhydramine hydrochloride, lorazepam, and metoclopramide hydrochloride in portable infusion-pump reservoirs, *Am.J.Hosp.Pharm.*, **1994**, *51*, 514–517.

SAMPLE
Matrix: formulations
Sample preparation: Ointment. Add pentane:EtOH 75:25 to ointment, sonicate for 20 min, dilute an aliquot to 100 mL with MeOH, allow to settle. Centrifuge and filter an aliquot of the supernatant, inject an aliquot of the filtrate. Cream, lotion. Stir cream or lotion in EtOH:THF:water 25:25:50 at 40° for 15 min, cool in an ice bath. Centrifuge and filter an aliquot of the supernatant, inject an aliquot of the filtrate. Gel. Dissolve gel in EtOH, sonicate, filter, inject an aliquot.

HPLC VARIABLES
Guard column: present but not specified (?)
Column: 250 × 2.1 10 μm Bondapak C18
Mobile phase: MeCN:water 48:52 containing 0.65% acetic acid, pH 3.18 (At the end of each day flush guard column only with MeOH:THF 75:25 for 30 min.)

Flow rate: 1
Injection volume: 20
Detector: UV 251

CHROMATOGRAM
Retention time: 3.67

OTHER SUBSTANCES
Simultaneous: bamipine lactate, beclomethasone dipropionate, betamethasone-17-valerate, hydrocortisone-21-acetate

KEY WORDS
ointment; creams; lotions; gels

REFERENCE
Kountourellis,J.E.; Markopoulou,C.K.; Ebete,K.O.; Stratis,J.A. Separation and determination of some corticosteroids combined with bamipine in pharmaceutical formulations by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 3507–3517.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:100 mM pH 4.5 NaH_2PO_4 15:85
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 2.6
Internal standard: propylparaben (3.3)

OTHER SUBSTANCES
Simultaneous: granisetron, methylprednisolone

KEY WORDS
injections; 5% dextrose; saline; water

REFERENCE
Pinguet,F.; Rouanet,P.; Martel,P.; Fabbro,M.; Salabert,D.; Astre,C. Compatibility and stability of granisetron, dexamethasone, and methylprednisolone in injectable solutions, *J.Pharm.Sci.*, **1995**, *84*, 267–268.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 2.36

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: liposomal preparations
Sample preparation: Dilute 1000-fold with MeOH/water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeOH:1% acetic acid 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.12

OTHER SUBSTANCES
Interfering: flumethasone

REFERENCE
Devoisselle,J.-M.; Vion-Dury,J.; Confort-Gouny,S.; Coustaut,D.; Cozzone,P.J. Liposomes containing fluorinated steroids: an analysis based on photon correlation and fluorine-19 nuclear magnetic resonance spectroscopy, *J.Pharm.Sci.*, **1992**, *81*, 249–254.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Extract 500 μL microsomal incubation with 3 mL ethyl acetate and 3 mL diethyl ether, evaporate the extracts to dryness, reconstitute the residues in 150 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Nucleosil 5C18
Column: 250 × 4.6 5 μm Nucleosil 5C8
Mobile phase: MeCN:pH 3.0 water 25:75
Flow rate: 1
Detector: Radioactivity, Radiomatic A250 Flo-One β (Canberra-Packard); UV 243; MS, Quattro II (Fisons Biotech MS, England) tandem quadrupole, nebulizing and drying gas nitrogen, electrospray, capillary voltage 4000V, cone voltage 30, 50 or 70 V, photomultiplier voltage 540 V, m/z 100 to 650 or m/z 100 to 850

CHROMATOGRAM
Retention time: 45

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
radiolabeled

REFERENCE

Gentile,D.M.; Tomlinson,E.S.; Maggs,J.L.; Park,B.K.; Back,D.J. Dexamethasone metabolism by human liver in vitro, metabolite identification and inhibition of 6-hydroxylation, *J.Pharmacol.Exp.Ther.*, **1996**, *277*, 105–112.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 1.0
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 2.9

OTHER SUBSTANCES

Simultaneous: diethylstilbestrol, hexestrol

REFERENCE

Nascimento,E.S.; Salvadori,M.C.; Ribeiro-Neto,L.M. Determination of synthetic estrogens in illegal veterinary formulations by HPTLC and HPLC, *J.Chromatogr.Sci.*, **1996**, *34*, 330–333.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-p (Nacalai Tesque, Japan)
Mobile phase: MeOH:water 60:40
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

OTHER SUBSTANCES

Simultaneous: prednisolone

REFERENCE

Tsuji,Y.; Kakegawa,H.; Miyataka,H.; Nishiki,M.; Matsumoto,H.; Satoh,T. Pharmaceutical properties of freeze-dried formulations of egg albumin, several drugs and olive oil, *Biol.Pharm.Bull.*, **1996**, *19*, 636–640.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 13.9 (dexamethasone acetate)

OTHER SUBSTANCES

Simultaneous: cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, 17α-hydroxyprogesterone, estradiol, estrone, progesterone

REFERENCE

Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 34–38.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.2 5 μm Ultrasphere C18
Mobile phase: Gradient. A was MeCN containing 1 mg/mL heptanesulfonic acid. B was 50 mM pH 2.2 phosphoric acid containing 1 mg/mL heptanesulfonic acid. A:B 12.5:87.5 for 2.5 min, to 48.5:51.5 over 13.5 min, maintain at 48.5:51.5 for 4 min
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: diphenhydramine, hydromorphone, creatinine, methyl paraben, propyl paraben, degradation products

KEY WORDS

stability-indicating; buffer

REFERENCE

Walker,S.E.; DeAngelis,C.; Iazzetta,J.; Eppel,J.G. Compatibility of dexamethasone sodium phosphate with hydromorphone hydrochloride or diphenhydramine hydrochloride, *Am.J.Hosp.Pharm.*, **1991**, *48*, 2161–2166.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal,

chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 4 mL water then 3 mL MeOH. Add aqueous steroid solution to cartridge, elute with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Limit of detection: 120 ng/mL

OTHER SUBSTANCES
Also analyzed: betamethasone 17-valerate, flumethasone 21-acetate

KEY WORDS
SPE

REFERENCE
Valenta,C.; Janout,H. Corticosteroid analysis by HPLC with increased sensitivity by use of precolumn concentration, *J.Liq.Chromatogr.*, **1994**, *17*, 1141–1146.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Vydac C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 95:5 to 65:35 over 9 min.
Column temperature: 40
Flow rate: 1
Detector: UV (wavelength not given)

OTHER SUBSTANCES
Simultaneous: atenolol

REFERENCE
Rubas,W.; Cromwell,M.E.M.; Shahrokh,Z.; Villagran,J.; Nguyen,T.-N.; Wellton,M.; Nguyen,T.-H.; Mrsny,R.J. Flux measurements across Caco-2 monolayers may predict transport in human large intestinal tissue, *J.Pharm.Sci.*, **1996**, *85*, 165–169.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM
Retention time: 9.3

Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: diethylstilbestrol, medroxyprogesterone, melengestrol acetate, trenbolone, triamcinolone acetonide, zeranol

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE
Matrix: urine
Sample preparation: Add 6 mL pH 9.2 ammonium buffer to 60 mL urine. Extract with 60 mL dichloromethane:EtOH 95:5. Agitate for 3 min, centrifuge at 900 g for 10 min. Filter organic layer through a qualitative filter paper, evaporate to dryness at 40° under a stream of nitrogen. Dissolve the residue in 8 mL solution supplied with immunoaffinity columns kit according to supplier's protocol (Randox Laboratories, UK). Resuspend the final residue in 50 μL mobile phase. Inject a 25 μL aliquot. (Buffer was 100 mL ammonium hydroxide and 200 g ammonium chloride in 500 mL water.)

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS-Hypersil
Mobile phase: MeCN:water 30:70
Flow rate: 1.0
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 5.9
Limit of detection: 4 ng/mL

KEY WORDS
SPE; horse

REFERENCE
Neto,L.M.R.; Salvadori,M.C.; Spinosa,H.S. Immunoafinity chromatography in the detection of dexamethasone in equine urine, *J.Chromatogr.Sci.*, **1997**, *35*, 549–551.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 in, filter through a 0.22 μm filter unit. Dilute 0.75-3mL urine to 4 mL with water. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH:10 mM NaOH 30:70, twice with 3 mL water and with 3 mL MeOH:10 mM HCl 30:70. Elute with 3 mL EtOH. Evaporate eluate under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 24:76
Flow rate: 0.5
Injection volume: 100

Detector: UV 254

CHROMATOGRAM
Retention time: 22.01

OTHER SUBSTANCES
Extracted: cortisone, hydrocortisone

KEY WORDS
dexamethasone is IS; SPE; human; pig

REFERENCE
Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 µL 5 µg/mL IS in MeOH, inject 20 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeCN:water 32:68
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

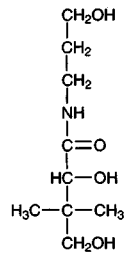
CHROMATOGRAM
Retention time: 10
Internal standard: methylprednisolone (9)

OTHER SUBSTANCES
Simultaneous: triamcinolone, triamcinolone acetonide, prednisone, prednisolone, corticosterone, hydroxyprogesterone, fluorocortisone acetate, cortisone, hydrocortisone, fluorocortisone
Interfering: betamethasone

KEY WORDS
SPE also discussed

REFERENCE
Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Solvent and solid-phase extraction of natural and synthetic corticoids in human urine, *J.Chromatogr.B*, **1994**, *652*, 83–89.

Dexpanthenol



Molecular formula: $C_9H_{19}NO_4$
Molecular weight: 205.25
CAS Registry No.: 81-13-0
Merck Index: 2988

SAMPLE

Matrix: formulations
Sample preparation: Dissolve an amount of formulation containing 10-20 mg dexpanthenol in 10 mL 0.5 M HCl, heat at 85 ± 2° for 30 min to hydrolyze dexpanthenol to aminopropanol. Remove an aliquot containing 1-2 mg dexpanthenol and add it to 10 mL 0.4 mg/mL fluorescamine in MeCN, add 2 mL ϵ-aminocaproic acid (concentration 60% of that of dexpanthenol) in mobile phase, make up to 25 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 Chromegabond C18
Mobile phase: MeOH:100 mM borate buffer adjusted to pH 8.0 ± 0.1 with 2 M NaOH 30:70
Flow rate: 1
Injection volume: 20
Detector: F ex 390 em 475-490 or UV 390

CHROMATOGRAM

Retention time: 24
Internal standard: ϵ-aminocaproic acid (12)

KEY WORDS

derivatization

REFERENCE

Umagat,H.; Tscherne,R. High performance liquid chromatographic determination of panthenol in bulk, premix, and multivitamin preparations, *Anal.Chem.*, **1980**, *52*, 1368–1370.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out amount containing about 1 mg dexpanthenol, add 50 mL 500 mM HCl, heat at 85° for 30 min, cool, centrifuge. Remove 1 mL of the supernatant and add it to 10 mL 400 μg/mL fluorescamine, dilute to 25 mL with 1% sodium borate, inject an aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 18:82
Detector: F ex 390 em 475-490

CHROMATOGRAM

Retention time: 7

KEY WORDS

derivatization; requires at least 100 μg/g dexpanthenol; liquid multivitamin product

REFERENCE

Hudson,T.S.; Subramanian,S.; Allen,R.J. Determination of pantothenic acid, biotin, and vitamin B12 in nutritional products, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 994–998.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.1 PRP-1
Mobile phase: MeCN:0.1 N perchloric acid 2.5:97.5
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: niacinamide, pyridoxine, thiamine

REFERENCE

Hamilton HPLC Applications Handbook, **1993**,

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 mm long 5 μm Nucleosil C18
Mobile phase: MeOH:10 mM pH 2.8 phosphate buffer 10:90
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM

Retention time: 5.2

OTHER SUBSTANCES

Simultaneous: pantolactone, pantoic acid, degradation products

REFERENCE

Gharehbagh,R.K.; Ebel,S. Stabilitätsanalytik von Dexpanthenol, 1.: HPLC-Bestimmung von Dexpanthenol, Pantolacton und Pantosäure [Stability analysis of dexpanthenol, 1.: Determination of dexpanthenol, pantolactone and pantoic acid by HPLC], *Pharmazie*, **1995**, *50*, 39–40.

Dextran

Molecular weight: (polymeric)
CAS Registry No.: 9004-54-0
Merck Index: 2989

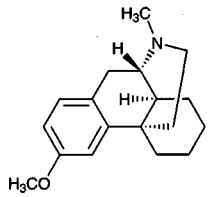
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.8 PolySep-GFC-P4000
Mobile phase: water
Flow rate: 0.8
Injection volume: 20
Detector: RI

CHROMATOGRAM
Retention time: 9-13 depending on average MW

REFERENCE
Phenomenex Catalog, **1994**, 1.091.

Dextromethorphan



Molecular formula: $C_{18}H_{25}NO$
Molecular weight: 271.40
CAS Registry No.: 125-71-3(dextromethorphan (d-form)), 125-69-9 (dextromethorphan HBr), 6700- 34-1 (dextromethorphan HBr monohydrate), 510-53-2 (racemethorphan)
Merck Index: 8274
Lednicer No.: 1 293

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 500 µL 4000 U/mL β-glucuronidase in 200 mM pH 5.0 acetate buffer, mix, incubate in water bath at 37° for 15 h. Inject a 100 µL aliquot onto column A and elute to waste with mobile phase A. After 5 min elute contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4 10 µm Hypersil CPS; B 20 × 4.6 5 µm Spherisorb CN + 250 × 4.6 5 µm Spherisorb CN
Mobile phase: A MeCN:water 5:95; B MeCN:triethylamine:water 20:0.06:79.94 adjusted to pH 2.5 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 100
Detector: F ex 220 em 305

CHROMATOGRAM

Retention time: 11
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: β-glucuronidase

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Hartter,S.; Baier,D.; Dingemanse,J.; Ziegler,G.; Hiemke,C. Automated determination of dextromethorphan and its main metabolites in human plasma by high-performance liquid chromatography and column switching, *Ther.Drug Monit.*, **1996**, *18*, 297–303.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL 200 mg Isoelute ODS SPE cartridge (Jones Chromatography) with 2 mL MeOH and 2 mL MeOH:water 3:97 at 20 mL/min. Add 1 mL plasma to the SPE cartridge at 0.5 mL/min, wash with 2 mL MeOH:water 3:97 and 2 mL MeOH:water 25:75 at 20 mL/min, elute with 4 mL MeOH:water:triethylamine:formic acid 80:20:0.5:0.32 at 4 mL/min, evaporate the eluate on a TurboVap evaporator at 35° using 172 kPa nitrogen, reconstitute the residue in 100 mL (sic) MeOH:water 10:90, inject an aliquot of the solution.

HPLC VARIABLES

Column: 50 × 2.1 3.5 µm Symmetry C18 (Waters)

Mobile phase: MeOH:water:formic acid 60:40:0.1
Flow rate: 0.3
Injection volume: 10
Detector: MS, Thornhill PE-Sciex API III-plus, TurboProbe 580°, nitrogen 7.5 L/min, nebulizer pressure 300 kPa, generating potential 4000 and 75 V, m/z 272-147

CHROMATOGRAM
Retention time: ≤ 2
Limit of detection: 50 pg/mL

KEY WORDS
pharmacokinetics; plasma; SPE

REFERENCE
Eichold,T.H.; Quijano,M.; Seibel,W.L.; Cruze,C.A.; Dobson,R.L.M.; Wehmeyer,K.R. Highly sensitive high-performance liquid chromatographic-mass spectrometric method for the analysis of dextromethorphan in human plasma, *J.Chromatogr.B*, **1997**, *698*, 147–154.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 2.4
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide,

oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: haloperidol (reduced), propranolol

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Retention time: 4.03

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine;

carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Dilute urine 3:1 or more with water. Vortex 1 mL CSF, plasma, or diluted urine with 100 μL 100 ng/mL IS, add 500 μL saturated sodium carbonate, mix, add 5 mL hexane containing 0.1% n-octylamine. Vortex for 60 s, centrifuge at 2000 g for 10 min. Re-extract aqueous phase with 5 mL hexane containing 0.1% n-octylamine, evaporate the combined hexane extracts to dryness under a stream of nitrogen in a 50° water bath. Reconstitute residue with 150 μL 100 mM HCl, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm CN

Column: 220 × 4.6 5 μm Brownlee Spheri-5CN (Applied Biosystems, USA)

Mobile phase: MeCN:n-octylamine:water 19:0.05:80.95 adjusted to pH 2.8 with phosphoric acid

Column temperature: 40

Flow rate: 1

Injection volume: 100

Detector: F ex 230 em 330

CHROMATOGRAM

Retention time: 10.7

Internal standard: levallorphan (7.4)

Limit of detection: 500 pg/mL

Limit of quantitation: 1 ng/mL (CSF, plasma), 5 ng/mL (urine)

OTHER SUBSTANCES

Extracted: dextrorphan

KEY WORDS

plasma

REFERENCE

Kimiskidis,V.K.; Kazis,A.D.; Niopas,I. Simultaneous determination of dextromethorphan and dextrorphan in human plasma, urine and cerebrospinal fluid by HPLC with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1267–1275.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Add 100 μL 300ng/mL IS, 100 μL 28% ammonium hydroxide and 5 mL n-butanol:hexane 10:90 to 5 mL urine, 1 mL plasma or 3 mL saliva. Rotate for 30 min and centrifuge at 4500 rpm for 10 min. Remove upper organic layer and extract it with 300 μL 100 mM HCl by vortexing for 20 min, centrifuge for 5 min. Inject a 40 μL (urine) or 200 μL (plasma, saliva) aliquot of the acidic layer.

HPLC VARIABLES

Column: Zorbax RP-phenyl

Mobile phase: MeCN:10 mM potassium phosphate 50:50, adjusted to pH 4.0 with 8.5% phosphoric acid

Column temperature: 40

Flow rate: 1.0

Injection volume: 40-200

Detector: F ex 280 em 310

CHROMATOGRAM

Retention time: 13.0

Internal standard: levallorphan (8.6)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Hu,O.Y.-P.; Tang,H.-S.; Lane,H.-Y.; Chang,W.-H.; Hu,T.-M. Novel single-point plasma or saliva dextromethorphan method for determining CYP2D6 activity, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 955–960.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μL 1 μg/mL pholcodine in water + 500 μL saturated sodium carbonate, mix, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 5 min, inject a 10-50 μL aliquot of the aqueous layer. Urine. 500 μL Urine + 50 μL 50 μg/mL pholcodine in water + 500 μL saturated sodium carbonate, mix, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 5 min, inject a 10-50 μL aliquot of the aqueous layer. (If desired, hydrolyse 500 μL plasma or urine with 500 μL 8000 U/mL β-glucuronidase (Helix pomatia, type H-1, Sigma) in 200 mM pH 5 acetate buffer at 37° for 16 h, proceed as above.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb cyano

Mobile phase: MeCN:water:triethylamine 17:82.94:0.06, adjusted to pH 3.0 with orthophosphoric acid

Flow rate: 1

Injection volume: 10-50

Detector: F ex 230 em 330

CHROMATOGRAM

Retention time: 10.5

Internal standard: pholcodine (6.3)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, levorphanol
Noninterfering: acetaminophen, clofibrate, codeine, cyclophosphamide, diclofenac, digoxin, doxepin, doxorubicin, estrogens, flucloxacillin, folic acid, furosemide, metformin, metoclopramide, miconazole, minoxidil, morphine, nifedipine, nitroglycerin, norcodeine, norethisterone, oxazepam, oxethazaine, prednisolone, pseudoephedrine, quinine, spironolactone, temazepam, tolbutamide, warfarin

KEY WORDS

plasma

REFERENCE

Chen,Z.R.; Somogyi,A.A.; Bochner,F. Simultaneous determination of dextromethorphan and three metabolites in plasma and urine using high-performance liquid chromatography with application to their disposition in man, *Ther.Drug Monit.*, **1990**, *12*, 97–104.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.312

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations
Sample preparation: 3 mL Sample + 5 mL 200 mg/mL o-dinitrobenzene in 1:1 MeOH:water, dilute to 50 mL with 1:1 MeOH:water, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water:ammonium formate buffer 45:54:1 (Ammonium formate buffer was 34 mL 28-30% ammonia diluted with 30 mL water. 30 mL 98% Formic acid was added, Caution! Exothermic! When cool this mixture (pH 3.9) was diluted to 100 mL with water.)
Flow rate: 2
Injection volume: 15
Detector: UV 280

CHROMATOGRAM
Retention time: 20
Internal standard: o-dinitrobenzene (11)

OTHER SUBSTANCES
Simultaneous: acetaminophen, guaifenesin, p-aminophenol

KEY WORDS
cough syrup

REFERENCE
McSharry,W.O.; Savage,I.V.E. Simultaneous high-pressure liquid chromatographic determination of acetaminophen, guaifenesin, and dextromethorphan hydrobromide in cough syrup, *J.Pharm.Sci.*, **1980**, *69*, 212–214.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL syrup to 50 mL with mobile phase, filter (0.45 μm), inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:water:formic acid:methanesulfonic acid 500:500:1:1, pH adjusted to 3.5 with 10% NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: guaifenesin, saccharin, benzoic acid

KEY WORDS
syrup

REFERENCE
Chen,T.M.; Pacifico,J.R.; Daly,R.E. High-pressure liquid chromatographic assay of dextromethorphan hydrobromide, guaifenesin, and sodium benzoate in an expectorant syrup, *J.Chromatogr.Sci.*, **1988**, *26*, 636–639.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, dissolve in 100 mL 100 mM pH 5.0 acetate buffer, let sit for 1 h with occasional mixing, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax SCX
Mobile phase: MeCN:30 mM KH_2PO_4 50:50
Flow rate: 2
Injection volume: 50
Detector: UV 263

CHROMATOGRAM
Retention time: 13.4

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, pseudoephedrine

KEY WORDS
tablets

REFERENCE
Murtha,J.L.; Julian,T.N.; Radebaugh,G.W. Simultaneous determination of pseudoephedrine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide by second-derivative photodiode array spectroscopy, *J.Pharm.Sci.*, **1988**, *77*, 715–718.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 10 mL to 50 mL with water.

HPLC VARIABLES
Column: 250 × 4.6 Whatman 10 μm PXS SCX
Mobile phase: MeOH:100 mM $(NH_4)H_2PO_4$, apparent pH 6.2
Column temperature: 40
Flow rate: 2
Injection volume: 20
Detector: UV 263

CHROMATOGRAM
Retention time: 5.37

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine

KEY WORDS
liquid formulations; stability-indicating

REFERENCE
Wilson,T.D.; Jump,W.G.; Neumann,W.C.; San Martin,T. Validation of improved methods for high-performance liquid chromatographic determination of phenylpropanolamine, dextromethorphan, guaifenesin and sodium benzoate in a cough-cold formulation, *J.Chromatogr.*, **1993**, *641*, 241–248.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out an amount of powdered tablets, syrup, or drops containing 300 mg dextromethorphan, add 75 mL mobile phase, extract mechanically for 7 min, make up to 100 mL with mobile phase, filter. Remove a 1 mL aliquot of the filtrate and add it to 2 mL 2 mg/mL labetalol in mobile phase, make up to 20 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:40 mM pH 4.3 acetate buffer 75:25
Flow rate: 1.5
Injection volume: 20
Detector: UV 278

CHROMATOGRAM
Retention time: 4.46
Internal standard: labetalol

KEY WORDS
tablets; syrup; drops

REFERENCE
Abdel-Moety,E.M.; Al-Deeb,O.A.; Khattab,N.A. Determination of dextromethorphan hydrobromide in bulk form and dosage formulations by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 4127–4134.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 13.1

OTHER SUBSTANCES
Simultaneous: bromhexine, chlorpheniramine, codeine, diphenhydramine, ephedrine, papaverine, phenylephrine

KEY WORDS
syrup; indirect conductometric detection; presence of compound causes a decrease in mobile phase conductivity

REFERENCE
Lau,O.-W.; Mok,C.-S. High-performance liquid chromatographic determination of active ingredients in cough-cold syrups with indirect conductometric detection, *J.Chromatogr.A*, **1995**, *693*, 45–54.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL MeOH:water:zinc sulfate 50:45:5 + 10 μM levallorphan in MeOH, centrifuge at 10000 g for 3 min, extract the supernatant twice with 3 mL dichloromethane. Combine dichloromethane extracts, dry under a stream of nitrogen, dissolve the residue in 200 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm polymer C18 (Astec, Whippany, NJ)
Mobile phase: MeCN:50 nM (sic) pH 9.0 ammonium carbonate buffer 60:40 (Buffer was adjusted to pH 9.0 with ammonium hydroxide.)
Column temperature: 30

Flow rate: 0.7
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 15.58
Internal standard: levallorphan (11.35)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: dextrorphan

KEY WORDS
liver

REFERENCE
Vielnascher,E.; Spatzenegger,M.; Mayrhofer,A.; Klinger,P.; Jäger,W. Metabolism of dextromethorphan in human liver microsomes: a rapid HPLC assay to monitor cytochrome P450 2D6 activity, *Pharmazie*, **1996**, *51*, 586–588.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 600 μL Microsomal incubation + 600 μL saturated sodium carbonate, place on ice, add 150 μL 12.5 μg/mL betaxolol, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 300 μL 0.5% orthophosphoric acid, extract, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 5 mm i.d. 4 μm Nova-Pak phenyl radial-Pak
Mobile phase: MeCN:MeOH:0.05% orthophosphoric acid 24:10:66
Flow rate: 1.6
Detector: F ex 261 em 306

CHROMATOGRAM
Retention time: 17
Internal standard: betaxolol
Limit of detection: 700 nM

OTHER SUBSTANCES
Extracted: metabolites, levorphanol (dextrorphan)

KEY WORDS
rat; liver

REFERENCE
Laslett,T.J.; Alvarez,F.; Nation,R.L.; Evans,A.M.; Scott,S.D.; Stupans,I. Effect of cyclophosphamide administration on the activity and relative content of hepatic P4502D1 in rat, *Xenobiotica*, **1995**, *25*, 1031–1039.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58

Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.14

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine,

phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.47 (A), 5.92 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine,

promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Mix 5 mL urine with 1 mL 200 mM pH 5 acetate buffer, 50 μL 600 mM sodium azide (Caution! Sodium azide is highly toxic!), 75 μL β-glucuronidase (1000 U/10 μL in 200 mM pH 5 acetate buffer). Mix, hydrolyze at 37° for 18 h, add 1 mL glycine buffer, 160 μL 50 ng/μL codeine, and 160 μL 50 ng/μL ethylmorphine. Add 6 mL hexane:ethyl acetate 50:50, rock for 15 min, centrifuge at 700 g for 5 min. Remove the organic phase, add another 6 mL hexane:ethyl acetate 50:50 to the aqueous layer, rock for 15 min, centrifuge at 700 g for 5 min. Combine the organic layers, add 1 mL 100 mM HCl, rock for 15 min, centrifuge at 700 g for 5 min. Discard the upper organic layer and add 1 mL glycine buffer to the aqueous layer. Mix for 5 s, add 8 mL hexane-ethyl acetate 50:50, rock for 15 min, centrifuge at 700 g for 5 min, evaporate the organic phase to dryness under nitrogen. Reconstitute the residue with 150 μL mobile phase, mix for 15 s, inject a 5-120 μL aliquot. (Prepare glycine buffer by mixing equal volumes of 1 M glycine containing 1 M NaCl and 1 M NaOH, adjust pH to 11.3 with either 1 M glycine containing 1 M NaCl or 1 M NaOH.)

HPLC VARIABLES
Guard column: 7 μm cyano (Bioanalytical Systems)
Column: 100 × 8 Nova-Pak CN HP Radial-Pak cartridge
Mobile phase: MeCN:buffer 12:88 (Buffer was 100 mM sodium acetate containing 600 μL triethylamine/L, adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-120
Detector: F ex 190 for 7 min, ex 235 for 8 min, return to ex 190 (no emission filter used)

CHROMATOGRAM
Retention time: 17
Internal standard: codeine (5), ethylmorphine (7)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Jones,D.R.; Gorski,J.C.; Hamman,M.A.; Hall,S.D. Quantification of dextromethorphan and metabolites: a dual phenotypic marker for cytochrome P450 3A4/5 and 2D6 activity, *J.Chromatogr.B*, **1996**, *678*, 105–111.

SAMPLE
Matrix: urine
Sample preparation: Add 500 μL saturated sodium carbonate to 500 μL urine, vortex, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1 (Caution! Chloroform is a carcino-

gen!), rotate at 36 rpm for 10 min, centrifuge at 2200 g for 5 min. Transfer the organic layer to a tube containing 100 μL 100 mM HCl, rotate at 36 rpm for 10 min, centrifuge at 2200 g for 5 min, inject a 5 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:triethylamine:water 6:0.12:93.88, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 1.3
Injection volume: 5
Detector: F ex 275 em 302

CHROMATOGRAM
Retention time: 5.0
Internal standard: pholcodine (2.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: caffeine

REFERENCE
Hoskins,J.M.; Shenfield,G.M.; Gross,A.S. Modified high-performance liquid chromatographic method to measure both dextromethorphan and proguanil for oxidative phenotyping, *J.Chromatogr.B*, **1997**, *696*, 81–87.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 1 mL 100 mM pH 5 acetate buffer + 20 μL β-glucuronidase/β-arylsulfatase (Helix pomatia, Boehringer Mannheim) + 50 μL 600 mM sodium azide in water, heat at 37° for 12 h. Inject a 100 μL aliquot onto column A and elute to waste at 0.5 mL/min, after 3 min elute contents of column A onto column B at 1.4 mL/min, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 4 10 μm LiChrosorb CN; B 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 50:50, adjusted to pH 4 with phosphoric acid
Flow rate: A 0.5; B 1.4
Injection volume: 100
Detector: UV 200 or F ex 280 em 310

CHROMATOGRAM
Retention time: 14
Limit of detection: <40 ng/mL (UV)

OTHER SUBSTANCES
Extracted: metabolites, levorphanol

KEY WORDS
column-switching

REFERENCE
Motassim,N.; Decolin,D.; Le Dinh,T.; Nicolas,A.; Siest,G. Direct determination of dextromethorphan and its three metabolites in urine by high-performance liquid chromatography using a precolumn switching system for sample clean-up, *J.Chromatogr.*, **1987**, *422*, 340–345.

SAMPLE
Matrix: urine

Sample preparation: Condition a Bond Elut silica modified with carboxylic acid ion-exchange groups SPE cartridge with 1 mL MeCN:100 mM HCl 40:60 and 1 mL water. Adjust 1 mL urine to pH 5.0-5.5, add β-glucuronidase/arylsulfatase (Helix pomatia (Boehringer Mannheim), heat at 37° for 18 h, add 1 mL to the SPE cartridge, wash with 1 mL water, wash with 500 μL 100 mM HCl, elute with 1 mL MeCN:100 mM HCl 40:60, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES

Column: 250 mm long 5 μm Zorbax phenyl
Mobile phase: MeCN:100 mM KH_2PO_4 45:55, adjusted to pH 4
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 310 or UV 280

CHROMATOGRAM

Retention time: 8.6
Limit of detection: 100 ng/mL (UV), 30 ng/mL (F)

OTHER SUBSTANCES

Extracted: metabolites, levorphanol

KEY WORDS

SPE

REFERENCE

Jacqz-Aigrain,E.; Menard,Y.; Popon,M.; Mathieu,H. Dextromethorphan phenotypes determined by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1989**, *495*, 361–363.

SAMPLE

Matrix: urine
Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 6 mL MeOH, 6 mL water, and 4 mL 100 mM pH 9.2 sodium carbonate buffer. 750 μL Urine + 750 μL 100 mM pH 5.0 sodium acetate buffer containing 20 μL β-glucuronidase-arylsulfatase (Helix pomatia, 100000 Fisherman units/mL, Boehringer Mannheim) + 50 μL 600 mM sodium azide, heat at 37° for 18 h. 250 μL Hydrolysed urine + 100 μL 10 μg/mL levallorphan tartrate in water + 2 mL 100 mM pH 9.2 sodium carbonate, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN, elute with 3 mL MeOH:MeCN: 2% phosphoric acid 50:30:20. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot. (For low concentrations of dextromethorphan: 500 μL Hydrolysed urine + 100 μL 1 μg/mL levallorphan tartrate in water + 2 mL 100 mM pH 9.2 sodium carbonate, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN, elute with 3 mL MeOH:MeCN:2% phosphoric acid 50:30:20. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 120 μL aliquot.)

HPLC VARIABLES

Guard column: 15 × 3.2 RP-2 (Brownlee)
Column: 250 × 4.6 5 μm Zorbax phenyl
Mobile phase: MeCN:MeOH:10 mM pH 2.5 phosphate buffer containing 2.5 mM 1-octanesulfonic acid 27:13:60
Column temperature: 30
Flow rate: 1
Injection volume: 20-120
Detector: F ex 270 em 312

CHROMATOGRAM

Retention time: 15.0
Internal standard: levallorphan tartrate (9.5)

Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, levorphanol

KEY WORDS
SPE

REFERENCE
Wenk,M.; Todesco,L.; Keller,B.; Follath,F. Determination of dextromethorphan and dextrorphan in urine by high-performance liquid chromatography after solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 341–344.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 1.25 μg levallorphan + 250 μL 140 mM pH 5 sodium acetate buffer, mix, add 25 μL β-glucuronidase (glucurase, from bovine liver, 5000 U/mL, Sigma), heat at 37° overnight, add 1.5 mL pH 11.3 glycine buffer, add 6 mL hexane: butanol 90:10, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: Nucleosil 7 C6H5
Mobile phase: MeCN:MeOH:buffer 20:10:70 (Buffer was 10 mM phosphate buffer containing 2.5 mM sodium 1-octanesulfonate, adjusted to pH 2.5 with concentrated phosphoric acid.)
Flow rate: 1.3
Injection volume: 10
Detector: F ex 270 em 312

CHROMATOGRAM
Retention time: 14.5
Internal standard: levallorphan (9)

OTHER SUBSTANCES
Extracted: levorphanol (dextrorphan)

REFERENCE
Caslavska,J.; Hufschmid,E.; Theurillat,R.; Desiderio,C.; Wolfisberg,H.; Thormann,W. Screening for hydroxylation and acetylation polymorphisms in man via simultaneous analysis of urinary metabolites of mephenytoin, dextromethorphan and caffeine by capillary electrophoretic procedures, *J.Chromatogr.B*, **1994**, *656*, 219–231.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 5000 U β-glucuronidase in 1 M pH 5.0 sodium acetate buffer, heat at 37° for 18 h, add 500 μL saturated sodium carbonate, add 10 mL hexane:triethylamine 99.9:0.1. Remove the organic layer, dry, reconstitute the residue in 250 μL mobile phase, inject a 5-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Selectosil (Phenomenex)
Mobile phase: MeCN:10 mM pH 3.0 potassium phosphate buffer 30:70
Flow rate: 1
Injection volume: 5-50
Detector: F ex 280 em 305

CHROMATOGRAM

Limit of detection: 2 μM

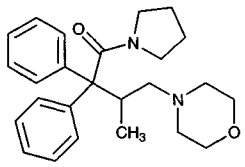
OTHER SUBSTANCES

Extracted: levorphanol

REFERENCE

Marinac,J.S.; Foxworth,J.W.; Willsie,S.K. Dextromethorphan polymorphic hepatic oxidation (CYP2D6) in healthy black american adult subjects, *Ther.Drug Monit.*, **1995**, *17*, 120–124.

Dextromoramide



Molecular formula: $C_{25}H_{32}N_2O_2$
Molecular weight: 392.54
CAS Registry No.: 357-56-2
Merck Index: 2997
Lednicer No.: 1 82

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 260

CHROMATOGRAM

Retention time: 7.05
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.835

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

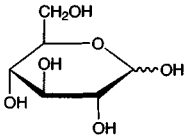
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Dextrose



Molecular formula: $C_6H_{12}O_6$

Molecular weight: 180.16

CAS Registry No.: 50-99-7, 77029-61-9 (D-glucopyranose monohydrate), 2280-44-6 (D-glucopyranose), 492-62-5 (α-D-glucopyranose), 492-61-5 (β-D-glucopyranose)

Merck Index: 4467

SAMPLE

Matrix: beverages, food

Sample preparation: Dilute beverages 10-fold, filter (0.45 μm), inject a 10 μL aliquot. Cereal, grain, tobacco. Pulverize 1 g cereal, grain, or tobacco, blend with 100 mL water for 10 min, filter, inject a 10 μL aliquot of the filtrate. Extract fatty samples with hexane: water 50:50, filter the aqueous layer, inject a 10 μL aliquot of the filtrate. Dilute dairy samples 100-fold, filter, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: H^+ guard column (Bio-Rad)

Column: Carbohydrate column in Pb^{2+} mode (Kratos)

Mobile phase: water

Column temperature: 85

Flow rate: 0.4

Injection volume: 10

Detector: UV 410 post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 1 mL reaction coil (ABI Analytical) at 100° to the detector. (Prepare the reagent as follows. Sonicate 2.5 g finely ground 4-aminobenzoic acid hydrazide and 2.46 mL concentrated HCl in 100 mL water until the mixture is homogeneous (Solution A). Sonicate 9.6 g NaOH and 1.46 g NaCl in 200 mL water until the mixture is homogeneous (Solution B). Combine Solution a and Solution B, sparge with helium, maintain at 0-5°.)

CHROMATOGRAM

Retention time: 21

Limit of detection: 20 ng

OTHER SUBSTANCES

Extracted: fructose, galactose, lactose, sucrose

KEY WORDS

post-column reaction; cereal; grain; tobacco; dairy products

REFERENCE

Femia,R.A.; Weinberger,R. Determination of reducing and non-reducing carbohydrates in food products by liquid chromatography with post-column catalytic hydrolysis and derivatization. Comparison with refractive index detection, *J.Chromatogr.*, **1987**, *402*, 127–134.

SAMPLE

Matrix: beverages, juice, milk

Sample preparation: Orange juice. Dilute orange juice 100-fold with water, filter (Millipore HV, 0.45 μm), dilute filtrate 10-fold, inject an aliquot. Beverages. Dilute soft drinks 1000-fold with water, inject an aliquot. Milk. Dilute 5 mL milk to 100 mL with mobile phase, filter (Millipore HV, 0.45 μm), dilute filtrate 50-fold, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Cation H (Bio-Rad)

Column: 300 × 3.8 9 μm HPX 87-H Aminex (Bio-Rad)
Mobile phase: 10 mM Sulfuric acid
Column temperature: 50
Flow rate: 0.5
Injection volume: 40
Detector: E following post-column reaction, Hewlett-Packard 1049A programmable electrochemical detector, Metrohm detector cell, cuprous oxide working electrode +550 mV, glassy carbon auxiliary electrode, Ag/AgCl (3 M KCl) reference electrode. The column effluent mixed with 200 mM NaOH pumped at 0.4 mL/min, the mixture flowed through a 220 × 0.8 single-bead string reactor packed with 0.6 mm glass beads to the detector. (Prepare cuprous oxide electrode as follows. Stir 300 mg conductive carbon cement (Gerhard Neubauer, Münster), 60 mg cuprous oxide (Fluka), and 300 μL acetone until a thick paste forms as the acetone evaporates. Pack conductive carbon cement into the base of a 3 mm diameter cavity carbon paste electrode base (Metrohm), allow to dry, polish with dry emery paper (grade 2/0, Oakey), remove surface layer with an acetone-soaked tissue, pack the paste into the cavity, allow to dry overnight, polish with dry emery paper (grade 2/0), 3 μm imperial micro finishing film sheet (3M), 0.3 μm imperial micro finishing film sheet (3M), and 0.05 μm alumina particles on a Buehler pad, sonicate for 2 min in water (Anal. Chim. Acta 1995, 300, 5).)

CHROMATOGRAM

Retention time: 10.47
Limit of detection: 1 μM

OTHER SUBSTANCES

Also analyzed: arabinose, cellobiose, fructose, fucose, galactitol, galactose, galacturonic acid, lactose, lactulose, lyxose, maltose, mannitol, mannose, myo-inositol, raffinose, rhamnose, ribose, sorbose, sucrose, xylose

KEY WORDS

orange juice; soft drinks; post-column reaction; fruit

REFERENCE

Huang,X.; Pot,J.J.; Kok,W.T. Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode, *Chromatographia*, **1995**, *40*, 684–689.

SAMPLE

Matrix: beverages, plants
Sample preparation: Beverages. Dilute 50-fold, filter (0.22 μm), inject an aliquot of the filtrate. Plants. Heat 1 g barley leaves and 10 mL EtOH:water 80:20 at 100° in a sealed tube for 15-30 min. Evaporate the liquid phase to dryness, reconstitute with water, pass through Analytichem trimethylaminopropyl and cyclohexyl SPE cartridges, inject an aliquot.

HPLC VARIABLES

Column: 300 × 6.5 Sugar-Pak I (Waters)
Mobile phase: water
Column temperature: 70
Flow rate: 0.4
Injection volume: 10
Detector: F ex 360 em 470 following post-column reaction. The effluent from the column passed through a 75 × 3.8 reactor containing Dowex 50 W × 2 sulfonic-acid type styrene divinylbenzene copolymer at 100° and mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min. This mixture flowed through a 530 μL reaction coil (Varian PCR-1) at 100° to the detector.

CHROMATOGRAM

Retention time: 18.27

Limit of detection: 60 pmole

OTHER SUBSTANCES
Extracted: fructose, sucrose

KEY WORDS
barley; SPE

REFERENCE
Coquet,A.; Haerdi,W.; Degli Agosti,R.; Veuthey,J.-L. Determination of sugars by liquid chromatography with post-column catalytic derivatization and fluorescence detection, *Chromatographia*, **1994**, *38*, 12–16.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL MeOH, shake for 1 min, centrifuge at 10000 rpm for 1 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4 Aminex A-27
Mobile phase: 500 mM Boric acid adjusted to pH 8.7 with KOH
Flow rate: 2
Injection volume: 10
Detector: F ex 357 (low-pressure mercury lamp) em 436 (420 nm cutoff filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.8 mm ID stainless steel coil at 150° to the detector. (Reagent was 20 g boric acid and 20 g ethanolamine in 1 L water.)

CHROMATOGRAM
Retention time: 27
Limit of quantitation: 5 nmoles

OTHER SUBSTANCES
Extracted: fructose, galactose, maltose, ribose, xylose

KEY WORDS
post-column reaction; serum

REFERENCE
Kato,T.; Kinoshita,T. Fluorometric detection and determination of carbohydrates by high-performance liquid chromatography using ethanolamine, *Anal.Biochem.*, **1980**, *106*, 238–243.

SAMPLE
Matrix: blood
Sample preparation: Freeze dry 40 μL serum, reconstitute with 40 μL cold mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 6 μm Shodex RSPak DC-613 sulfonated polystyrene 55% cross-linked with divinylbenzene calcium form (Showa Denko)
Mobile phase: MeCN:water 70:30
Column temperature: 4
Flow rate: 0.9
Injection volume: 20
Detector: UV 280 following post-column reaction. The column effluent mixed with 500 mM pH 8.5 borate buffer pumped at 0.5 mL/min and 1% 2-cyanoacetamide in water pumped

at 0.5 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° and a 1 m × 0.5 mm PTFE cooling coil to the detector.

CHROMATOGRAM

Retention time: 10 (β-anomer), 11 (α-anomer)

KEY WORDS

post-column reaction; serum

REFERENCE

Honda,S.; Suzuki,S.; Kakehi,K. Improved analysis of aldose anomers by high-performance liquid chromatography on cation-exchange columns, *J.Chromatogr.*, **1984**, *291*, 317–325.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 10 μL 1 μM L-fucose + 400 μL 500 mM trichloroacetic acid, centrifuge at 4° at 1500 g for 5 min. Remove a 100 μL aliquot of the supernatant and add it to 100 μL 1 M pH 11.0 borate buffer, mix, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 TSK gel Sugar AXG trimethylammonium-bonded styrene-divinylbenzene copolymer resin strong anion-exchange (Tosoh)

Mobile phase: 1 M pH 9.0 Borate buffer

Column temperature: 60

Flow rate: 0.4

Injection volume: 100

Detector: F ex 330 em 460 following post-column reaction. 600 mM NaOH pumped at 0.15 mL/min mixed with 15 mM meso-1,2-bis(4-methoxyphenyl)ethylenediamine in EtOH:water 30:70 pumped at 0.15 mL/min and the mixture flowed through a 1 m × 0.5 mm ID stainless steel coil. This mixture mixed with the column effluent and the mixture flowed through a 20 m × 0.5 mm ID stainless steel coil at 140° and a 1 m × 0.5 mm ID air-cooled stainless steel coil to the detector. (Prepare meso-1,2-bis(4-methoxyphenyl)ethylenediamine as follows. Heat 105 g benzil and 122 g salicaldehyde in 750 mL EtOH at 60° until they dissolve, introduce a weak stream of ammonia with stirring over 3 h, cool, filter, wash the precipitate with EtOH, dry under vacuum at 80°. Suspend 192 g of this product in 500 mL acetic anhydride, reflux for 14 h using a constant temperature bath at 148-150°, the product (O,O',N,N'-tetraacetyl-meso-1,2-bis(2-hydroxyphenyl)ethylenediamine) crystallized on slow cooling, filter, wash the solid with a little acetic anhydride. Suspend the product in 250 mL 42% hydrobromic acid:acetic acid 50:50 and reflux for 3 h. Filter off the product that separates on cooling and dissolve it in 400 mL hot water, neutralize with 20% NaOH. Filter off the product that separates and recrystallize it from MeCN to give meso-1,2-bis(2-hydroxyphenyl)ethylenediamine as a colorless powder (mp 184-186°). Mix 2.44 g meso-1,2-bis(2-hydroxyphenyl)ethylenediamine with 20 mmole p-anisaldehyde in 100 mL MeCN, reflux until the reaction is complete (at least 1 h), reduce the reaction mixture to half its volume by distillation, cool, filter. Recrystallize the product from toluene to give N,N'-disalicylidene-meso-1,2-bis(4-methoxyphenyl)ethylenediamine (mp 202-207°) (formed via a diaza-Cope rearrangement). Suspend 5 mmole N,N'-disalicylidene-meso-1,2-bis(4-methoxyphenyl)ethylenediamine in 50 mL 2 M sulfuric acid and steam distil until no more salicaldehyde comes over, filter the reaction mixture while it is hot, make the filtrate strongly basic (pH 11) with 20% NaOH. Filter off the crystalline product and recrystallize it from MeCN to give meso-1,2-bis(4-methoxyphenyl)ethylenediamine (mp 151-152°) (Chem. Ber 1976, 109, 1).)

CHROMATOGRAM

Retention time: 28.0

Internal standard: L-fucose (22.2)

Limit of detection: 520-560 nM

OTHER SUBSTANCES
Extracted: 2-deoxy-D-glucose, 2-deoxy-D-ribose, L-rhamnose

KEY WORDS
post-column reaction; serum; rat; pharmacokinetics

REFERENCE
Umegae,Y.; Nohta,H.; Ohkura,Y. Simultaneous determination of 2-deoxy-D-glucose and D-glucose in rat serum by high-performance liquid chromatography with post-column fluorescence derivatization, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 963–965.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 250 μg/mL melibiose, mix, add 200 μL ice cold 35 mg/mL 5-sulfosalicylic acid, let stand on ice for 20 min, centrifuge at 9000 g for 5 min, mix with Amberlite IR-120 H^+:Amberlite IRA 400 Cl^- 40:60, centrifuge, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Carbopac PA-1 (Dionex)
Column: 250 × 40 Carbopac PA-1 (Dionex)
Mobile phase: 160 mM NaOH containing 0.675 mM zinc acetate (At the end of each plasma sample wash with 1 M NaOH for 4 min.)
Flow rate: 1
Injection volume: 25
Detector: E, Dionex pulsed electrochemical detector, detection potential -0.01 V (0-0.5 s), oxidation potential +0.75 V (0.51-0.64 s), reduction potential -0.75 V (0.65-0.75 s), integration period 0.05-0.5 s

CHROMATOGRAM
Retention time: 3.2
Internal standard: melibiose (4.0)

OTHER SUBSTANCES
Extracted: lactulose, 3-O-methylglucose, mannitol

KEY WORDS
plasma

REFERENCE
Fleming,S.C.; Kynaston,J.A.; Laker,M.F.; Pearson,A.D.J.; Kapembwa,M.S.; Griffin,G.E. Analysis of multiple sugar probes in urine and plasma by high-performance anion-exchange chromatography with pulsed electrochemical detection. Application in the assessment of intestinal permeability in human immunodeficiency virus infection, *J.Chromatogr.*, **1993**, *640*, 293–297.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 25 μg adonitol + 1 mL EtOH, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to 500 μL under a stream of nitrogen. Apply the residue to a Bond Elut SCX SPE cartridge (100 mg/mL), elute with 2 mL water. Apply the eluate to a Bond Elut SAX SPE cartridge (100 mg/mL), elute with 2 mL water. Freeze-dry the eluate, reconstitute in 100 μL MeOH:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 10 μm Gelpack GL-C64Z sulfonated styrene-divinylbenzene copolymer with zinc ions (Hitachi-Kasei)

Mobile phase: MeCN:water 80:20
Column temperature: 80
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M-1000S quadrupole, negative-ion APCI, drift voltage -25 V, vaporizer 250°, desolvator 399°, m/z 215, $[M + Cl]^-$, SIM The column effluent was mixed with MeOH:chloroform 99:1 pumped at 0.5 mL/min, the combined effluent then flowed to the MS detector.

CHROMATOGRAM
Retention time: 8
Internal standard: adonitol (m/z 187) (9)

OTHER SUBSTANCES
Extracted: 1,5-anhydroglucitol, arabinose, arabitol, erythritol, fructose, galactose, mannitol, myoinositol, sorbitol, xylitol

KEY WORDS
serum; SPE

REFERENCE
Niwa,T.; Tohyama,K.; Kato,Y. Analysis of polyols in uremic serum by liquid chromatography combined with atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1993**, *613*, 9–14.

SAMPLE
Matrix: blood
Sample preparation: 70 µL Serum + 10 µL 1 mM D-glucosamine.HCl + 20 µL 1 M K_2HPO_4 + 10 µL benzoyl chloride + 25 µL 8 M NaOH, vortex at 2500 vibrations/min for 5 min, add 10 µL 1.4 M phosphoric acid and 100 µL ethyl acetate, vortex at 2500 vibrations/min for 1 min. Remove 25 µL of the ethyl acetate phase and add it to 100 µL MeCN: water 70:30, inject an aliquot.

HPLC VARIABLES
Guard column: 5 µm Kromasil 100 C18
Column: 250 × 4 5 µm Kromasil 100 C18
Mobile phase: Gradient. MeCN:water from 70:30 to 95:5 over 30 min.
Flow rate: 1
Injection volume: 50
Detector: UV 228 or MS, electrospray, Finnigan MAT, TSQ 700, flow rate 1 µL/min, 2.8 kV, drying gas 140

CHROMATOGRAM
Retention time: 7.5, 7.9, 8.1 (tetrabenzoyl), 15.6, 15.9 (pentabenzoyl)
Internal standard: D-glucosamine (9.7)
Limit of detection: 1-5 pmol

OTHER SUBSTANCES
Extracted: benzyl alcohol, adenosine, mannitol, 2-desoxy-D-glucose, cytidine, myoinositol, sucrose

KEY WORDS
serum; derivatization; fetal bovine serum

REFERENCE
Oehlke,J.; Brudel,M.; Blasig,I.E. Benzoylation of sugars, polyols and amino acids in biological fluids for high-performance liquid chromatographic analysis, *J.Chromatogr.B*, **1994**, *655*, 105–111.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Plasma + 190 μL 50 mM NaOH, filter (Advantec Q0100, 10000 molecular mass cut-off), inject a 50 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 40 × 4 5 μm TSKgel SAX (Cl type) (Showa Denko, Tokyo)

Mobile phase: 50 mM NaOH containing 30 mM sodium butyrate (Protect mobile phase from carbon dioxide absorption with a soda lime guard column. At the start of the day wash column with 500 mM NaOH at 0.5 mL/min for 20 min and with mobile phase at 0.7 mL/min for 10 min.)

Column temperature: 40

Flow rate: 0.7

Injection volume: 50

Detector: Chemiluminescence following post-column reaction. The mobile phase flowed through an immobilized pyranose oxidase reactor and a co-immobilized 3-hydroxybutyrate dehydrogenase/NADH oxidase reactor at 40°. The effluent from the reactors mixed with 2 mM luminol in 400 mM pH 10 carbonate buffer pumped at 0.5 mL/min and 10 mM potassium ferricyanide in water pumped at 0.5 mL/min and the mixture flowed to the detector. (Store luminol solution in a refrigerator for 1 day before use. Prepare immobilized pyranose oxidase reactor as follows. Circulate 10 mL 1 mg/mL pyranose oxidase (EC 1.1.3.10 from Polyporus obtusus, 10 U/mg, Takara Shuzo, Kyoto) in 50 mM pH 7.0 phosphate buffer through a 40 × 4 column of tresylated poly(vinyl alcohol) beads at 0.2 mL/min for 4 h. Prepare a co-immobilized 3-hydroxybutyrate dehydrogenase/NADH oxidase reactor as follows. Circulate 10 mL 0.5 mg/mL 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30, Pseudomonas sp., grade III, 120 U/mg) in 50 mM pH 7.0 phosphate buffer containing 0.5 mg/mL NADH oxidase (from Bacillus megaterium, 50 U/mg, Toyobo, Osaka) through a 40 × 4 column of tresylated poly(vinyl alcohol) beads at 0.2 mL/min for 4 h. Prepare the columns of poly(vinyl alcohol) beads as follows. Wash 1 g 13 μm poly(vinyl alcohol) beads (GS-520, Showa Denko, Tokyo) with 50 mL dry acetone, suspend in 20 mL dry acetone:pyridine 50:50 with vigorous stirring, add 1 mL 2,2,2-trifluoroethanesulfonyl chloride dropwise over 2 min, stir for 10 min, wash beads with 10 mL acetone, wash beads with 20 mL 1 mM HCl, slurry pack in a 40 × 4 column (J. Chromatogr.A 1996, 724, 354).)

CHROMATOGRAM

Retention time: 3

Limit of detection: 2 μg/mL

OTHER SUBSTANCES

Extracted: 1,5-anhydroglucitol, 3-hydroxybutyrate

KEY WORDS

post-column reaction; plasma; ultrafiltrate; immobilized enzyme reactor

REFERENCE

Kiba,N.; Saegusa,K.; Furusawa,M. Post-column enzyme reactors for chemiluminometric detection of glucose, 1,5-anhydroglucitol and 3-hydroxybutyrate in an anion-exchange chromatographic system, *J.Chromatogr.B*, **1997**, *689*, 393–398.

SAMPLE

Matrix: blood, erythrocytes, tissue

Sample preparation: Homogenize lens tissue in 4 (human) or 1 (rat) mL 2 mg/mL sodium fluoride. Dilute 600 μL frozen and thawed erythrocytes with 400 μL water. Filter (Amicon Centrifree) 1 mL homogenate, plasma, or diluted erythrocytes while centrifuging at 2400 g for 30 min. 200 μL Filtrate + 10 μL 1 mg/mL IS, mix, lyophilize, add 200 μL 100 mg/mL p-nitrobenzoyl chloride in pyridine, heat at 60° for 1 h, add 1 drop of water, add 2

mL chloroform. Wash mixture twice with 2 mL 5% sodium bicarbonate and twice with 3 mL 1 M HCl by vortexing for 1 min and centrifuging for 30 s, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 13
Internal standard: perseitol (α-mannoheptitol) (26)
Limit of detection: 1-2 ng

OTHER SUBSTANCES
Extracted: fructose, myo-inositol, sorbitol, mannitol

KEY WORDS
plasma; lens; human; rat; normal phase; derivatization

REFERENCE
Petchey,M.; Crabbe,M.J.C. Analysis of carbohydrates in lens, erythrocytes, and plasma by high-performance liquid chromatography of nitrobenzoate derivatives, *J.Chromatogr.*, **1984**, *307*, 180–184.

SAMPLE
Matrix: bulk
Sample preparation: Evaporate hydrolysates of glycosaminoglycans to dryness, reconstitute in 500 μL 10% benzoic anhydride in pyridine containing 5% 4-dimethylaminopyridine, heat at 37° for 1.5 h, add 4.5 mL water, shake vigorously, pass through a Sep-Pak C18 SPE cartridge three times, wash with 10 mL pyridine:water 10:90, wash with 5 mL water, reverse the direction of flow and elute with 2.5 mL MeCN, evaporate the eluate to dryness, reconstitute with MeCN, centrifuge at 11000 g for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 RP-18
Column: 250 × 4.6 Supelcosil LC-18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 12.2

OTHER SUBSTANCES
Simultaneous: N-acetylgalactosamine, N-acetylglucosamine, 1,6-anhydroidose, fucose, galactosamine, glucosamine, mannose, 1-methylfucose, 1-methylgalactose, 1-methylglucose, 1-methylmannose, 1-methylxylose, xylose
Interfering: galactose

KEY WORDS
derivatization; SPE

REFERENCE

Karamanos,N.K.; Hjerpe,A.; Tsegenidis,T.; Engfeldt,B.; Antonopoulos,C.A. Determination of iduronic acid and glucuronic acid in glycosaminoglycans after stoichiometric reduction and depolymerization using high-performance liquid chromatography and ultraviolet detection, *Anal.Biochem.*, **1988**, *172*, 410–419.

SAMPLE

Matrix: bulk

Sample preparation: Sample (solid) + 50 μL 500 mM 3-methyl-1-phenylpyrazolone in MeOH + 50 μL 300 mM NaOH, heat at 70° for 30 min, cool, neutralize with 100 mM HCl, evaporate to dryness, add 200 μL water, add 200 μL chloroform, shake vigorously. Remove the aqueous layer and evaporate it to dryness, reconstitute in 0.1-1 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Capcell Pak C18 (Shiseido)

Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 18:82

Flow rate: 1

Injection volume: 20

Detector: UV 245 or E, Irika E-502, glassy carbon working electrode 600 mV, AgCl reference electrode

CHROMATOGRAM

Retention time: 23

Limit of detection: 0.1 pmole (E), 1 pmole (UV)

OTHER SUBSTANCES

Simultaneous: mannose, lyxose, rhammose, N-acetylglucosamine, N-acetylgalactosamine, galactose, arabinose, fucose

KEY WORDS

derivatization

REFERENCE

Honda,S.; Akao,E.; Suzuki,S.; Okuda,M.; Kakehi,K.; Nakamura,J. High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl-5-pyrazolone derivatives, *Anal.Biochem.*, **1989**, *180*, 351–357.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 10 μg of a reducing sugar in 100 μL 0.01% perchloric acid in acetic anhydride, let stand for 5 min, add 100 μL EtOH, let stand at room temperature for 30 min, add 1 mg potassium carbonate, add 100 μL dichloromethane, centrifuge. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL dichloromethane, add 200 μL 33% HBr in acetic acid, let stand for 1 h, concentrate under reduced pressure below 60°, dissolve the residue in 100 μL acetone, add an excess of (-)-2-tert-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid, add an equimolar amount of potassium bicarbonate, heat at 60° for 1 h, purify an aliquot by TLC (50 × 50 silica gel on aluminum sheet (Merck DC-Alufolien) developed with toluene:ethyl acetate 2:1), remove the fluorescent band at R_f 0.5, extract with MeCN, inject an aliquot of the extract.

HPLC VARIABLES

Column: 150 × 4.6 ODS

Mobile phase: MeCN:water:isopropanol 40:40:10

Flow rate: 0.8

Detector: F

CHROMATOGRAM
Retention time: 28.5 (L), 30.1 (D)
Limit of detection: "a few pmoles"

OTHER SUBSTANCES
Simultaneous: arabinose, fucose, galactose, mannose, rhamnose, xylose

KEY WORDS
derivatization; chiral

REFERENCE
Nishida,Y.; Bai,C.; Ohrui,H.; Meguro,H. A highly sensitive method to identify the DL-configurations of monosaccharides based on (-)-TBMB carboxylic acid and HPLC, *J.Carbohydr.Chem.*, **1994**, *13*, 1003–1008.

SAMPLE
Matrix: carbohydrates
Sample preparation: Mix 10 nmoles total monosaccharides with 200 μL 2 M trifluoroacetic acid, flush with nitrogen for a few min, seal, heat at 100° for 6 h, evaporate to dryness under reduced pressure in a desiccator over NaOH pellets, reconstitute with water, inject an aliquot.

HPLC VARIABLES
Column: 80 × 8 11 μm Hitachi No. 2633 resin (quaternary ammonium)
Mobile phase: Gradient. A was 250 mM pH 8.2 borate buffer. B was 400 mM pH 7.4 borate buffer. C was 600 mM pH 9.3 borate buffer. A:B:C from 100:0:0 to 0:100:0 over 15 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 10 min, maintain at 0:0:100.
Column temperature: 65
Flow rate: 1
Injection volume: 20
Detector: F ex 331 em 383 following post-column reaction. The column effluent mixed with 10% 2-cyanoacetamide in water pumped at 0.25 mL/min and 600 mM pH 9.3 borate buffer pumped at 0.25 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100 ± 0.5° to the detector.

CHROMATOGRAM
Retention time: 65
Limit of detection: 0.1-1 nmole

OTHER SUBSTANCES
Simultaneous: arabinose, fucose, galactose, lyxose, mannose, rhamnose, ribose, xylose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Takahashi,M.; Kakehi,K.; Ganno,S. Rapid, automated analysis of monosaccharides by high-performance anion-exchange chromatography of borate complexes with fluorimetric detection using 2-cyanoacetamide, *Anal.Biochem.*, **1981**, *113*, 130–138.

SAMPLE
Matrix: food
Sample preparation: Freeze chewing gum, pulverize. Sonicate 1 g with 80 mL EtOH: water 96:4 at 60° for 20 min, cool, filter, rinse the filter, make up the filtrate to 100 mL. Remove a 5 mL aliquot and evaporate it to dryness under reduced pressure, add 4 mL pyridine, add 500 μL benzoyl chloride, sonicate at 60° for 1 h with swirling every 15 min, add 500 μL MeOH, swirl, let stand for 10 min, add 50 mL water (Z. Lebensm. Unters.

Forsch. 1984, 178, 199), shake, add to a Sep-Pak RP-18 SPE cartridge (conditioning of cartridge is not necessary), rinse flask four times with 5 mL portions of water, add the rinses to the SPE cartridge, push 5 mL volumes of air through cartridge 3 times, elute with five 10 mL portions of isooctane:ether:MeCN 60:32:8, make up the volume of the eluate to 50 mL, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3 5 μm LiChrosorb Si 60 (glass column)
Mobile phase: Isooctane:ether:MeCN 150:60:10
Flow rate: 0.9
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 13
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: mannitol, saccharose, sorbitol, xylitol

KEY WORDS
derivatization; SPE; chewing gum; mayonnaise; normal phase

REFERENCE
Galensa,R. Hochleistungs-flüssigchromatographische Bestimmung von Zuckeralkoholen mit UV-Detektion im ppm-Bereich in Lebensmitteln. I. [High-performance liquid chromatographic determination of sugar alcohols with UV-detection in the ppm-range in food. I.], *Z.Lebensm.Unters.Forsch.*, **1983**, *176*, 417–420.

SAMPLE
Matrix: formulations
Sample preparation: Remove the water from 10 μL syrup under reduced pressure for 10 min, reconstitute with 2 mL pyridine. Remove a 25 μL aliquot and add it to 75 μL reagent, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure at room temperature, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject an aliquot. (Prepare reagent by dissolving 100 mg 4-nitrobenzoyl chloride in pyridine with gentle warming.)

HPLC VARIABLES
Column: 150 × 3 5 μm LiChrosorb SI 60
Mobile phase: n-Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water
Flow rate: 1.4
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: fructose, glycerin, propylene glycol, saccharose, sorbitol

KEY WORDS
syrup; derivatization; normal phase

REFERENCE
Nachtmann,F.; Budna,K.W. Sensitive determination of derivatized carbohydrates by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *136*, 279–287.

SAMPLE
Matrix: fruit
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeCN. 15 g Freeze-dried avocado powder + 150 mL hexane, allow to stand for 30 min, filter (coarse sintered glass), wash solid with another 50 mL hexane and dry it under gentle suction. Add 85 mL water to the solid and mix it to a fine paste, add 250 mL water, let stand for 1 h, filter (coarse sintered glass), wash the solid with three 100 mL portions of water. Concentrate the aqueous filtrate to less than 200 mL on a rotary evaporator at 50 mmHg and 60°, add 20 μL preservative per 1 mL of extract, make up to 200 mL. Pass 7 mL of this solution through the SPE cartridge, discard the first 2 mL of eluate, inject an aliquot of the remainder of the eluate. The preservative was 25 mg cycloheximide and 25 mg chloramphenicol in 20 mL water.)

HPLC VARIABLES
Column: μBondapak/carbohydrate
Mobile phase: MeCN:water 87.5:12.5
Flow rate: 1.1 for 20 min then 2.1 until perseitol elutes
Injection volume: 20
Detector: RI

CHROMATOGRAM
Retention time: 14.5

OTHER SUBSTANCES
Extracted: fructose, mannoheptulose, perseitol

KEY WORDS
SPE; avocado

REFERENCE
Shaw,P.E.; Wilson,C.W.,III; Knight,R.J.,Jr. High-performance liquid chromatographic analysis of D-manno-heptulose, perseitol, glucose, and fructose in avocado cultivars, *J.Agric.Food Chem.*, **1980**, *28*, 379–362.

SAMPLE
Matrix: glycoconjugates
Sample preparation: Mix 0.1-1.5 mg glycoconjugate with 200 μL 2 M trifluoroacetic acid, flush with nitrogen for a few min, seal, heat at 100° for 6 h, evaporate to dryness under reduced pressure in a desiccator over NaOH pellets, reconstitute with 200 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Hitachi No. 2633 resin
Mobile phase: Gradient. A was 250 mM pH 8.2 borate buffer. B was 400 mM pH 7.4 borate buffer. C was 600 mM pH 9.3 borate buffer. A:B:C from 100:0:0 to 0:100:0 over 15 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 11 min, maintain at 0:0:100.
Column temperature: 65 ± 1
Flow rate: 1
Injection volume: 20
Detector: UV 276 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide pumped at 0.5 mL/min and 600 mM pH 10.5 borate buffer pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100 ± 0.2° and a 1 m × 0.5 mm ID PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: 64
Limit of detection: 1 nmole

OTHER SUBSTANCES
Extracted: arabinose, fucose, galactose, lyxose, mannose, rhamnose, ribose, xylose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Takahashi,M.; Nishimura,Y.; Kakehi,K.; Ganno,S. Sensitive ultraviolet monitoring of aldoses in automated borate complex anion-exchange chromatography with 2-cyanoacetamide, *Anal.Biochem.*, **1981**, *118*, 162–167.

SAMPLE
Matrix: glycoproteins
Sample preparation: 200 μg Glycoprotein + 100 μL water + 100 μL 4 M trifluoroacetic acid, heat at 100° for 6 h, cool to room temperature, evaporate to dryness under reduced pressure at 35°, add 40 μL reagent, heat at 80° for 1 h, cool to room temperature, add 200 μL water, add 200 μL chloroform, vortex vigorously, centrifuge for 1 min, inject an aliquot of the upper aqueous layer. (Prepare the reagent by mixing 165 mg ethyl p-aminobenzoate, 35 mg sodium cyanoborohydride, 41 μL glacial acetic acid, and 350 μL glacial acetic acid.)

HPLC VARIABLES
Column: 150 × 3.9 Pico.Tag (Waters)
Mobile phase: MeCN:MeOH:50 mM pH 4.5 sodium acetate 4:2:94
Column temperature: 45
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Extracted: galactose, mannose

KEY WORDS
derivatization

REFERENCE
Kwon,H.; Kim,J. Determination of monosaccharides in glycoproteins by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1993**, *215*, 243–252.

SAMPLE
Matrix: juice
Sample preparation: Filter apple juice, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 6 20 μm DA X-4 anion-exchange (Durrum Chemical Co.)
Mobile phase: 700 mM Boric acid containing 7.5 mM ethylenediamine, adjusted to pH 8.6 with 8 M NaOH (Ethylenediamine was Nanochrome II from Breda Scientific, Holland.)
Column temperature: 78
Flow rate: 0.7
Injection volume: 20

Detector: F ex 360 (bandpass filter) em 455 (bandpass filter) following post-column reaction. The column effluent flowed through a 30 m × 0.5 mm ID PTFE coil at 145° to the detector.

CHROMATOGRAM
Retention time: 99
Limit of detection: 100-400 pmole

OTHER SUBSTANCES
Simultaneous: fructose, galactose, mannose, sucrose

KEY WORDS
post-column reaction; apple

REFERENCE
Mopper,K.; Dawson,R.; Liebezelt,G.; Hansen,H.-P. Borate complex ion exchange chromatography with fluorimetric detection for determination of saccharides, *Anal.Chem.*, **1980**, *52*, 2018–2022.

SAMPLE
Matrix: perfusate
Sample preparation: 200 μL Perfusate + 200 μL MeCN, centrifuge at 2000 rpm, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 110 × 4.6 5 μm irregular silica (HSCP, Bourne End, UK)
Mobile phase: MeCN:water:1,4-diaminobutane 67:33:0.03
Flow rate: 0.8
Injection volume: 20
Detector: UV 285 following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed to the detector. (Prepare stock reagent solution by dissolving 25 g cupric sulfate in 500 mL water, add 500 mL 28% ammonium hydroxide. Prepare working reagent by mixing 100 mL stock solution, 250 mL 28% ammonium hydroxide, and 650 mL water, filter (Whatman GF/C). Place the waste solvent bottle in a chemical fume hood.)

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 2.5 nmole

OTHER SUBSTANCES
Extracted: fructose, sucrose

KEY WORDS
post-column reaction

REFERENCE
Grimble,G.K.; Barker,H.M.; Taylor,R.H. Chromatographic analysis of sugars in physiological fluids by postcolumn reaction with cuprammonium: a new and highly sensitive method, *Anal.Biochem.*, **1983**, *128*, 422–428.

SAMPLE
Matrix: plants
Sample preparation: Condition trimethylaminopropylsilica SAX and cyclohexylsilica SPE cartridges (Analytichem) with 4 mL MeOH and 4 mL water. Heat 1 g plant material with 10 mL EtOH:water 80:20 in a sealed tube at 100° for 15-30 min, evaporate the extract to dryness, reconstitute with water, pass through the SPE cartridges, inject a 50 μL aliquot of the eluate.; SPE

HPLC VARIABLES

Column: 300 × 6.5 Sugar Pak-1 microparticulate gel, calcium form (Waters)
Mobile phase: 100 μM Calcium EDTA
Column temperature: 70
Flow rate: 0.4
Injection volume: 50
Detector: F ex 360 em 470 following post-column reaction. The column effluent mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min and the mixture flowed through a 530 μL reaction coil (Varian PCR1) at 100° to the detector.

CHROMATOGRAM

Retention time: 10.92
Limit of detection: 15.8-62.5

OTHER SUBSTANCES

Extracted: arabinose, fructose, fucose, galactose, lactose, mannose, rhamnose, xylose

KEY WORDS

post-column reaction; SPE

REFERENCE

Coquet,A.; Veuthey,J.-L.; Haerdi,W. Selective post-column fluorigenic reaction with benzamidine for trace level detection of reducing saccharides in liquid chromatography, *J.Chromatogr.*, **1991**, *553*, 255–263.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 160 × 4 8 μm DAX8 anion-exchange resin, sulfate form (Durrum Chemical Co.) (Regenerate resin outside the column by washing 10 g resin with 400 mL water, 400 mL 500 mM NaCl at 50°, water, 0.5 N sodium sulfate at 50° (until a negative chloride test is obtained), water, and EtOH:water 95:5. Slurry pack below 70° with mobile phase at 1.14 mL/min.)
Mobile phase: EtOH:water 87.6:12.4
Column temperature: 88
Flow rate: 0.57
Injection volume: 5-100
Detector: UV 562 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed for 5 min through a coil of 0.3 mm ID PTFE at 100° to the detector. (Prepare reagent by mixing equal volumes of solution A and solution B, the mixture is stable for at least 1 month. Solution A is 1 g of copper sulfate pentahydrate and 3.7 g aspartic acid in 1 L water. Solution B is 38 g sodium carbonate decahydrate and 2 g sodium bicinchoninate (Pierce Chemical Co.) in 1 L water.)

CHROMATOGRAM

Retention time: 168
Limit of detection: <500 pmole

OTHER SUBSTANCES

Simultaneous: arabinose, digitose, fructose, fucose, galactose, 2-d-galactose, 6-d-glucose, gulose, lyxose, mannose, 3-O-methylglucose, rhamnose, 2-d-ribose, ribose, sorbose, tagatose, xylose

KEY WORDS

post-column reaction

REFERENCE

Mopper,K. Improved chromatographic separations on anion-exchange resins. I. Partition chromatography of sugars in ethanol, *Anal.Biochem.*, **1978**, *85*, 528–532.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in water containing 5 mg/mL galactose, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 Aminex carbohydrate HPX-87 (BioRad)
Mobile phase: water
Column temperature: 80
Flow rate: 0.2
Injection volume: 20
Detector: RI

CHROMATOGRAM

Retention time: 5
Internal standard: galactose (7)

OTHER SUBSTANCES

Simultaneous: arabinose, arabitol, dextran, erythritol, fructose, fucose, galactan, galactitol, galactomannan, gentibiose, glycol, iditol, lyxose, maltose, maltotriose, mannitol, mannose, pentaerythritol, propylene glycol. pullulan, raffinose, rhamnose, ribitol, sorbitol, sorbose, sucrose, trehalose, xylitol, xylose
Noninterfering: xylan
Interfering: melibiose, turanose, melezitose

KEY WORDS

detector temp 30

REFERENCE

Dokladalova,J.; Barton,A.Y.; Mackenzie,E.A. High pressure liquid chromatographic determination of sorbitol in bulk sorbitol, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 664–666.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 11 μm Hitachi No. 2633 pellicular quaternary ammonium anion-exchange resin
Mobile phase: 200 mM pH 7.2 Borate buffer for 22 min then 500 mM pH 9.6 borate buffer
Column temperature: 65
Flow rate: 0.35
Detector: F ex 331 em 385 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100° to the detector. (Reagent was 2-cyanoacetamide in 300 mM pH 7.5 phosphate/borate buffer.)

CHROMATOGRAM

Retention time: 94

OTHER SUBSTANCES

Simultaneous: cellobiose, fructose, galactose, lactose, maltose, mannose, rhamnose, ribose, xylose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Matsuda,Y.; Takahashi,M.; Kakehi,K.; Ganno,S. Fluorimetric determination of reducing carbohydrates with 2-cyanoacetamide and application to automated analysis of carbohydrates as borate complexes, *Anal.Chem.*, **1980**, *52*, 1079–1082.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 MicroPak NH2 (Convert column to phosphate form by conditioning with 1% phosphoric acid.)
Mobile phase: MeCN:water 70:30
Flow rate: 0.8
Injection volume: 1
Detector: F ex 334 em (5-58 band filter) following post-column reaction. The column effluent mixed with 5% cyanoacetamide in 100 mM pH 10.4 potassium borate buffer pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.23 mm ID coil at 120° to the detector.

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: fructose, maltose, ribose

KEY WORDS
post-column reaction

REFERENCE
Schlabach,T.D.; Robinson,J. Improvements in sensitivity and resolution with the cyanoacetamide reaction for the detection of chromatographically separated reducing sugars, *J.Chromatogr.*, **1983**, *282*, 169–177.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.2 μm Micrometer Metricel), inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 300 × 6.5 Sugar PAK I (Waters)
Mobile phase: water
Column temperature: 90
Flow rate: 0.5
Injection volume: 10-20
Detector: RI

CHROMATOGRAM
Retention time: 9.18

OTHER SUBSTANCES
Simultaneous: raffinose, lactose, galactose

KEY WORDS
detector temp 75°

REFERENCE

Betschart,H.F.; Prenosil,J.E. High-performance liquid chromatographic analysis of the products of enzymatic lactose hydrolysis, *J.Chromatogr.*, **1984**, *299*, 498–502.

SAMPLE

Matrix: solutions

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeCN and 5 mL water. Mix 250 μL of an aqueous solution with 225 μL 1% dansylhydrazine in EtOH and 45 μL 10%trichloroacetic acid in water, heat at 65° for 20 min, dilute with water to an organic solvent concentration of ≤5%, add a 5 mL aliquot to the SPE cartridge, wash with 5 mL MeCN:water 5:95 at ≤2 mL/min, elute with 6 mL MeCN:water 20:80 at ≤2 mL/min (J. Chromatogr. 1983, 256, 27), lyophilize the eluate, reconstitute with MeCN:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm 600 RPB C18 (Alltech)

Mobile phase: MeCN:water 20:80 containing 10 mM formic acid, 40 mM acetic acid, and 1 mM triethylamine. (After each run flush column with MeCN:MeOH 20:80 for 5 min.)

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 9

Limit of detection: 200-300 pmole

OTHER SUBSTANCES

Simultaneous: fucose, galactose, lyxose, mannose, xylose

KEY WORDS

derivatization; SPE

REFERENCE

Eggert,F.M.; Jones,M. Measurement of neutral sugars in glycoproteins as dansyl derivatives by automated high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *333*, 123–131.

SAMPLE

Matrix: solutions

Sample preparation: Heat 100-200 pmole sample with 20 μL 4 M trifluoroacetic acid and 20 μL 4 M HCl in a tube sealed under vacuum at 100° for 6 h, add 500 pmole L-rhamnose, evaporate to dryness under reduced pressure at 50°, add 50 μL 9.8% sodium bicarbonate solution (freshly prepared), add 2 μL acetic anhydride, let stand at room temperature with occasional stirring for 30 min, add 200 μL 100-200 mesh Dowex 50W-X2 (H^+), check that pH is about 3. Add the mixture to a 100 × 5 column and wash it with 5 bed volumes of water, evaporate to dryness under reduced pressure, add 5 μL reagent, seal tube, heat at 100° for 13-15 min, add 2 μL 20 mg/mL sodium cyanoborohydride in water (freshly prepared), reseal the tube, heat at 90° for 8 h, dilute with 20 μL water, inject the whole amount on to a 600 × 7.5 10 μm TSK-GEL G2000PW column (Toyo Soda) and elute with 20 mM pH 7.5 ammonium acetate buffer at 0.5 mL/min, collect the sugar fraction at 40-55 min. Evaporate the eluate to dryness and reconstitute it with 250 μL water, inject a 5 μL aliquot. (Prepare reagent by mixing 500 mg 2-aminopyridine, 400 μL concentrated HCl, and 11 mL water.)

HPLC VARIABLES

Column: two 250 × 4.6 5 μm Ultrasphere-ODS column in series

Mobile phase: MeCN:250 mM pH 4.0 sodium citrate buffer 1:99

Flow rate: 0.5

Injection volume: 5
Detector: F ex 320 em 400

CHROMATOGRAM
Retention time: 36
Internal standard: L-rhamnose
Limit of quantitation: 10 pmoles

OTHER SUBSTANCES
Simultaneous: N-acetyl-D-mannosamine, N-acetylgalactosamine, N-acetylglucosamine, 2-deoxy-D-ribose, fucose, galactose, mannnose, ribose, xylose

KEY WORDS
derivatization; SPE

REFERENCE
Takemoto,H.; Hase,S.; Ikenaka,T. Microquantitative analysis of neutral and amino sugars as fluorescent pyridylamino derivatives by high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *145*, 245–250.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 80 × 3 Hitachi 2633
Mobile phase: 700 mM pH 8.5 Borate buffer containing 0.01% EDTA
Column temperature: 60
Flow rate: 0.7
Injection volume: 20
Detector: E, Irika E-502, glassy carbon working electrode 350 mV, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 100 mM ethylenediamine sulfate pumped at 0.25 mL/min and 700 mM pH 9.0 borate buffer pumped at 0.25 mL/min and the mixture flowed through a 30 m × 0.5 mm ID PTFE coil at 140° and a 10 m × 0.2 mm ID cooling coil to the detector.

CHROMATOGRAM
Retention time: 36
Limit of detection: 1 pmole

OTHER SUBSTANCES
Simultaneous: galactose, mannose, rhamnose, xylose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Enami,K.; Konishi,T.; Suzuki,S.; Kakehi,K. Use of ethylenediamine sulphate for post-column derivatization of reducing carbohydrates to electrochemically oxidizable compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *361*, 321–329.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: HPIC-AS6 anion-exchange (Dionex)
Mobile phase: 150 mM NaOH
Column temperature: 36

Flow rate: 0.5
Injection volume: 20
Detector: UV 500 following post-column reaction. The column effluent mixed with the reagent pumped at 0.2 mL/min (?) and the mixture flowed through a knitted 10 m × 0.3 mm ID PTFE coil at 90° and then a short knitted PTFE coil at 22° to the detector. (Reagent was 2 mg/mL thymol in concentrated sulfuric acid, let stand for 30 min after preparation, discard after 48 h. (The reagent was displaced from a pressure vessel into the post-column reaction system by pumping n-heptane into the vessel.)

CHROMATOGRAM
Retention time: 9.0
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: arabinose, desoxyribose, fructose, lactose, maltose, mannose, raffinose, ribose, saccharose, xylose
Noninterfering: methyl arabinose, methyl glycose, rhamnose, rutinose, trehalose
Interfering: galactose

KEY WORDS
post-column reaction

REFERENCE
Engelhardt,H.; Ohs,P. Trace analysis of sugars by HPLC and post-column derivatization, *Chromatographia*, **1987**, *23*, 657–662.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-NH_2
Mobile phase: MeCN:water 75:25
Column temperature: 22
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: fructose, glycerol, maltose, sucrose

REFERENCE
Johnson,J.M.; Harris,C.H. Selecting the most effective filtration media for HPLC analysis of saccharides, *J.Chromatogr.Sci.*, **1987**, *25*, 267–269.

SAMPLE
Matrix: solutions
Sample preparation: Add 50 μL 500 mM 3-methyl-1-phenyl-2-pyrazolin-5-one in MeOH and 50 μL 300 mM NaOH to 10-500 pmole saccharides, heat at 70° for 30 min, cool to room temperature, neutralize with 100 mM HCl, evaporate to dryness under reduced pressure, add 200 μL water, add 200 μL chloroform, shake vigorously. Remove the aqueous layer and evaporate it to dryness, reconstitute with 0.1-1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Capcell Pak C18 (Shiseido, Tokyo)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 18:82
Injection volume: 20
Detector: UV 245, E, Irika E-502, glassy carbon working electrode 600 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 21
Limit of detection: 1 pmole (UV), 100 fmole (E)

OTHER SUBSTANCES
Simultaneous: N-acetylgalactosamine, N-acetylglucosamine, arabinose, fucose, galactose, lyxose, mannose, rhamnose

KEY WORDS
derivatization

REFERENCE
Honda,S.; Akao,E.; Suzuki,S.; Okuda,M.; Kakehi,K.; Nakamura,J. High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl-5-pyrazolone derivatives, *Anal.Biochem.*, **1989**, *180*, 351–357.

SAMPLE
Matrix: solutions
Sample preparation: Inject 20-50 μL of an aqueous solution of 14C-labelled dextrose.

HPLC VARIABLES
Column: 300 × 3.9 Bondapak NH2
Mobile phase: MeCN:water 85:15
Injection volume: 20-50

KEY WORDS
Preparative; purification of radiolabelled compound; water

REFERENCE
Schleicher,E.D.; Vogt,B.W. Standardization of serum fructosamine assays, *Clin.Chem.*, **1990**, *36*, 136–139.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 50 mg sugars in 700 μL pyridine, add 700 μL 720 mM hydroxylamine hydrochloride in pyridine, heat at 60° for 10 min, add 250 μL acetic anhydride, heat at 75° for 10 min, evaporate to dryness under reduced pressure, reconstitute with 3 mL chloroform. Wash the organic layer three times with 6 mL portions of water and dry it over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, take up in chloroform, pass through silica gel using chloroform, evaporate the eluate to dryness, reconstitute, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm μBondapak C18
Mobile phase: Gradient. MeCN:water from 35:75 to 50:50 over 15 min.
Flow rate: 1
Injection volume: 5
Detector: UV 207

CHROMATOGRAM
Retention time: k' 3.9
Limit of detection: 3 μg

OTHER SUBSTANCES
Also analyzed: allose, altrose, arabinose, fructose, galactose, gulose, idose, lyxose, mannose, ribose, talose, xylose

KEY WORDS
derivatization

REFERENCE
Velasco,D.; Castells,J.; Lopez-Calahorra,F. High-performance liquid chromatographic separation of monosaccharides as their peracetylated ketoximes and aldononitriles, *J.Chromatogr.*, **1990**, *519*, 228–236.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 8.7 Aminex HPX-87C Ca^{++} (Bio-rad)
Mobile phase: Water
Column temperature: 50
Flow rate: 0.6
Detector: E, pulsed amperometric detector (Dionex ?), E1 0.1 V, T1 300 ms, E2 0.6 V, T2 120 ms, E3 -0.8 V, T3 300 ms, following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 10.02 (β), 11.22 (α)

OTHER SUBSTANCES
Also analyzed: 2-deoxygalactitol, 2-deoxyribositol, fucitol, fucose, galactitol, galactose, inositol, mannitol, mannose, perseitol, sorbitol

KEY WORDS
post-column reaction

REFERENCE
Wang,W.T.; Safar,J.; Zopf,D. Analysis of inositol by high-performance liquid chromatography, *Anal.Biochem.*, **1990**, *188*, 432–435.

SAMPLE
Matrix: solutions
Sample preparation: Add 55 μL phenylisocyanate to a 1 mg/mL solution in DMF, heat at 55° for 95 min, cool, add 500 μL MeOH, let stand for 5 min, make up to 6 mL with DMF, dilute an aliquot 10-fold with DMF, inject an aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm ODS 224 RP18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 15.7

Limit of detection: 0.2 ng

OTHER SUBSTANCES
Simultaneous: allose, arabinose, deoxyglucose, deoxyribose, fucose, galactose, lyxose, mannose, methylgalactoside, methylglucoside, methylmannoside, rhamnose, ribose, xylose

KEY WORDS
derivatization; more than one derivative was observed; retention time is for major derivative

REFERENCE
Rakotomanga,S.; Baillet,A.; Pellerin,F.; Baylocq-Ferrier,D. Liquid chromatographic analysis of monosaccharides with phenylisocyanate derivatization, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 587–591.

SAMPLE
Matrix: solutions
Sample preparation: Add 25 nmoles 3-O-methylglucose, evaporate the solution to dryness, add 50 μL 300 mM sodium cyanoborohydride in 2 M pH 7.0 ammonium acetate (freshly prepared), heat at 105° for 4 h, add 100 μL water, add 40 μL 6 M formic acid, evaporate to dryness under reduced pressure, add 500 μL MeOH, evaporate to dryness, repeat MeOH evaporation twice more, add 100 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness, add 100 μL EtOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under reduced pressure, reconstitute with 20 μL MeCN:water 60:40, add 180 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: Gradient. A was 50 mM pH 6.8 ammonium acetate. B was 100 mM pH 6.8 ammonium acetate in MeCN:MeOH:water 44:10:46. A:B 78:22 until the run is over, to 0:100 over 5 min, maintain at 0:100 for 6 min, return to initial conditions over 5 min
Column temperature: 30
Flow rate: 0.8
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: 3-O-methylglucose (20)
Limit of detection: 50 pmole

OTHER SUBSTANCES
Simultaneous: fucose, galactose, mannose, ribose, xylose

KEY WORDS
derivatization

REFERENCE
Spiro,M.J.; Spiro,R.G. Monosaccharide determination of glycoconjugates by reverse-phase high-performance liquid chromatography of their phenylthiocarbamyl derivatives, *Anal.Biochem.*, **1992**, *204*, 152–157.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: ION-300
Mobile phase: 2.5 mM sulfuric acid
Column temperature: 70

Flow rate: 0.4
Detector: RI

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: acetic acid, citric acid, EtOH, fructose, glycerol, lactic acid, malic acid, MeOH, tartaric acid

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REFERENCE
Keystone Scientific Catalog, 1993-4, p. 45.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.8 Aminex HPX-87P cation-exchange (Bio-Rad)
Mobile phase: Water
Column temperature: 85
Flow rate: 0.8
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 20 m × 0.3 mm ID PTFE coil at 90° and a coil at 0° to the detector. (Reagent was prepared from 4000 ppm Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) in 2 M NaOH (A) and 40 mM hydrogen peroxide (B). A:B 70:30.)

CHROMATOGRAM
Retention time: 15
Limit of detection: 70 ng

OTHER SUBSTANCES
Simultaneous: arabinose, fructose, galactose, mannose, ribose, xylose

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KEY WORDS
post-column reaction

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Hernandez,V.; Toribio,L.; Mendez,R. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) as a reagent for post-column derivatization of neutral monosaccharides in high pressure liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 1105–1116.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Shodex Sugar SP 0810P and SP 0810
Mobile phase: water
Column temperature: 80
Flow rate: 0.5
Detector: RI

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: arabinose, fructose, galactose, glycerol, lactose, lactulose, mannitol, pullulan P-10, raffinose, sorbitol, stachyose, sucrose, xylitol

REFERENCE
Majors,R.E. Polymeric liquid chromatography column technology in Japan, *LC.GC*, **1993**, *11*, 778–788.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.8 Sarasep CAR-Ca (MetaChem)
Mobile phase: 50 μg/mL dicalcium EDTA
Column temperature: 80
Flow rate: 0.7
Detector: RI

CHROMATOGRAM
Retention time: 9.6

OTHER SUBSTANCES
Simultaneous: acetone, acetonitrile, adonitol, arabinose, arabitol, cellobiose, erythritol, ethanol, ethylene glycol, fructose, fucose, galactinol, galactitol, galactopinitol, galactose, gentianose, gentiobiose, glucoheptose, glycerol, isomaltose, ketose, lactitol, lactose, lactulose, maltitol, maltose, maltotetraose, maltotriose, mannitol, mannoheptulose, mannose, melezitose, melibiose, methanol, myo-inositol, nystose, palatinol, palatinose, perseitol, pinitol, propylene glycol. raffinose, rhamnose, ribose, sedoheptulosan, sedoheptulose, sorbitol, sorbose, styachyose, sucrose, tagatose, trehalose, turanose, volemitol, xylitol, xylose

REFERENCE
MetaChem Catalog, **1994**, 65.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.1 PRP-X300 (Hamilton)
Mobile phase: 50 mM pH 4.5 NaH_2PO_4
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 5.3

OTHER SUBSTANCES
Simultaneous: gluconic acid, glucono-δ-lactone

REFERENCE
Baxter Scientific Products Catalog, 1990-1, p. 123, p. 123.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 75 × 4.6 2.8 μm quaternary ammonium-functionalized poly(styrene-divinylbenzene) in a PEEK column. (The poly(styrene-divinylbenzene) beads (Anal.Biochem. 1993, 212, 351), were nitrated, reduced to amines, and quaternized with iodomethane (J.Chromatogr.A 1994, 685, 213).)

Mobile phase: Gradient. A was 50 mM NaOH. B was 50 mM NaOH containing 500 mM sodium acetate. A:B 100:0 for 2 min, to 80:20 over 10 min. At the end of each run change mobile phase to 300 mM NaOH over 5 min, maintain at 300 mM NaOH for 15 min, re-equilibrate at initial conditions for 15 min.

Flow rate: 0.5

Injection volume: 10 (PEEK injection valve and sample loop)

Detector: E, Dionex PAD-2 pulsed amperometric detector, gold working electrode, Ag/AgCl reference electrode, E1 0.10 V (300 ms), E2 0.60 V (120 ms), E3 -0.60 V (300 ms)

CHROMATOGRAM

Retention time: 1.68

OTHER SUBSTANCES

Simultaneous: laminaritriose, maltopentaose, maltose, maltotetraose, maltotriose, panose, turanose

REFERENCE

Corradini,C.; Corradini,D.; Huber,C.G.; Bonn,G.K. High-performance anion-exchange chromatography of carbohydrates using a new resin and pulsed amperometric detection, *Chromatographia*, **1995**, *41*, 511–515.

SAMPLE

Matrix: solutions

Sample preparation: Filter (Whatman GF/F treated at 450° for 12 h) 3 L seawater, lyophilize filtrate, take up residue in 5 mL 1 M sulfuric acid, heat at 90° for 4 h, cool, neutralize with calcium carbonate (powder, previously treated at 450° for 12 h), sonicate, centrifuge at 3000 rpm for 5 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 50 × 4 CarboPac PA1 (Dionex)

Column: 250 × 4 CarboPac PA1 (Dionex)

Mobile phase: 15 mM NaOH. (After each analysis wash column with 300 mM NaOH for 5 min. Each week wash column with 600 mM NaOH for 20 min. NaOH solutions should be carbonate free, pass helium through mobile phase reservoirs to prevent carbonate formation.)

Flow rate: 1

Injection volume: 25

Detector: E, Dionex pulsed electrochemical detector, gold working electrode, Ag/AgCl reference electrode, E1 0.05 mV T1 480 ms, E2 +0.6 mV T2 120 ms, E3 -0.6 mV T3 70 ms following post-column reaction. The column effluent mixed with 380 mM NaOH pumped at 0.5 mL/min and this mixture flowed to the detector.

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Simultaneous: arabinose, fructose, fucose, galactose, glucosamine, mannose, rhamnose, ribose, xylose

KEY WORDS

seawater

REFERENCE
Kerhervé,P.; Charrière,B.; Gadel,F. Determination of marine monosaccharides by high-pH anion-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.A*, **1995**, *718*, 283–289.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.7 Hypercarb porous graphitic carbon (Shandon)
Mobile phase: 100 mM NaOH containing 0.15 mM phenol
Column temperature: 22
Flow rate: 1
Injection volume: 20
Detector: UV 287

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: galactose, fucose

KEY WORDS
indirect UV detection

REFERENCE
Lu,B.; Stefansson,M.; Westerlund,D. Indirect detection of saccharides in reversed-phase liquid chromatography with highly alkaline mobile phases, *J.Chromatogr.A*, **1995**, *697*, 317–327.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was hexane. B was ethyl acetate. C was 0.1% formic acid in MeCN. D was 0.1% formic acid in water. A:B:C:D 100:0:0:0 for 5 min, to 0:100:0:0 over 15 min, maintain at 0:100:0:0 for 5 min, to 0:0:100:0 over 5 min, maintain at 0:0:100:0 for 5 min; to 0:0:0:100 over 25 min, maintain at 0:0:0:100 for 5 min.
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM
Retention time: 44.46

OTHER SUBSTANCES
Simultaneous: acetylcholine, cholesterol, choline, cortisone, estradiol, glycine, phenylalanine, sodium, testosterone

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 CarboPac PA1 anion-exchange column (Dionex)
Mobile phase: 150 mM NaOH
Column temperature: 35
Flow rate: 0.7
Detector: E, Shimadzu L-ECD-6A, BAS thin-layer electrochemical cell, BAS Ag/AgCl/3 M NaCl reference electrode, 1 mm diameter copper oxide electrode (polish with 0.05 μm alumina for 5 min, wash, sonicate, heat in air at 100° for 3 h), 0.45 V

CHROMATOGRAM
Retention time: 4.4
Limit of detection: 0.1-0.2 pmole

OTHER SUBSTANCES
Simultaneous: arabinose, fructose, inositol, lactose, sorbitol, sucrose

REFERENCE
Kano,K.; Takagi,K.; Inoue,K.; Ikeda,T.; Ueda,T. Copper electrodes for stable subpicomole detection of carbohydrates in high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *721*, 53–57.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot of a 2 mg/mL aqueous solution.

HPLC VARIABLES
Column: 300 × 7.8 Aminex HPX-87C sulfonated polystyrene (Bio-Rad)
Mobile phase: water
Column temperature: 25
Flow rate: 0.8
Injection volume: 25
Detector: RI or UV 190

CHROMATOGRAM
Retention time: 5.68 (β), 6.58 (α)

OTHER SUBSTANCES
Also analyzed: fructose, galactose, maltose, mannose, α-methylglucoside

REFERENCE
Nishikawa,T.; Suzuki,S.; Kubo,H.; Ohtani,H. On-column isomerization of sugars during high-performance liquid chromatography: analysis of the elution profile, *J.Chromatogr.A*, **1996**, *720*, 167–172.

SAMPLE
Matrix: solutions
Sample preparation: Freeze dry a 10 μL of a 100 μM-10 mM solution in a glass tube, reconstitute with 50 μL 750 mM aminopyrazine in acetic acid, heat at 90° for 30 min, add 50 μL 700 mM borane-dimethylamine complex in acetic acid, heat at 90° for 5 min, cool to room temperature. Remove a 10 μL aliquot and dry under reduced pressure at 40° for 10 min to remove the acetic acid, reconstitute with 5 mL 700 mM pH 9.0 potassium borate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 PALPAK Type A (Takara Shuzo, Kyoto) [anion-exchange]
Mobile phase: MeCN:700 mM pH 9.0 borate buffer 10:90
Column temperature: 65
Flow rate: 0.3
Injection volume: 5

Detector: F ex 245 em 410

CHROMATOGRAM

Retention time: 40
Limit of detection: 200 fmole

OTHER SUBSTANCES

Simultaneous: N-acetylgalactosamine, N-acetylglucosamine, fucose, galactose, mannose, rhamnose, xylose

KEY WORDS

derivatization

REFERENCE

Tachiki,K.; Yoshida,H.; Hamase,K.; Zaitsu,K. Aminopyrazine as a precolumn derivatizing reagent for the fluorescence detection of monosaccharides in high-performance liquid chromatography, *Anal.Sci.*, **1997**, *13*, 509–512.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 50 μL aliquot of a 500 μM saccharide solution in MeCN:water 30:70 with 50 μL 10 mM reagent in MeCN and 100 μL 0.5% trichloroacetic acid in MeCN, heat at 65° in the dark for 3 h. Remove a 50 μL aliquot of the reaction mixture, add 200 μL water, add 200 μL ethyl acetate, mix, centrifuge at 3000 rpm for 2 min, repeat the ethyl acetate wash twice more. Dry the aqueous layer under reduced pressure, reconstitute with 200 μL MeCN, inject an aliquot. (Synthesis of reagent, R-(+)-DBD-ProCZ, is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro- 2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32

and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 mg DBD-F in 10 mL MeCN to 47 mg R-(+)-proline in 20 mL 250 mM pH 11.5 sodium carbonate solution, stir at room temperature for 30 min, wash with ethyl acetate, adjust the pH of the aqueous layer to 1-2 with 2 M HCl, extract three times with 30 mL ethyl acetate. Combine the extracts and evaporate them under reduced pressure, recrystallize from benzene/ethyl acetate to give R-(+)-4-(N,N-dimethylaminosulfonyl)-7-(2-carboxypyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro) as yellow needles (mp 187-9° d) (Analyst 1989, 114, 1233). Suspend 55 mg (R)-(+)-DBD-Pro in 55 mL anhydrous diethyl ether at 0°, add 110 mg phosphorus pentachloride, stir at 5° for 1 h, filter quickly, evaporate to dryness under reduced pressure, dry under vacuum over phosphorus pentoxide for 12 h to give R-(+)-4-(N,N-dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro-Cl) as yellow crystals (mp 116-17°) (Analyst 1993, 118, 759). Add 130 mg DBD-Pro-Cl dissolved in 25 mL anhydrous benzene dropwise to 100 mL MeOH containing 70 mg hydrazine hydrate, stir for 30 min at room temperature, evaporate under reduced pressure, recrystallize from ethyl acetate:MeOH 90:10 to give R-(+)-4-(2-carbazolylpyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (R-(+)-DBD-ProCZ) as orange crystals (mp 107-109°) (Anal. Proc. 1994, 31, 265).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water 15:85
Column temperature: 40
Flow rate: 1
Detector: F ex 450 em 540

CHROMATOGRAM

Retention time: 11.07

OTHER SUBSTANCES

Simultaneous: N-acetyl-D-glucosamine, arabinose, galactose, mannose, xylose

KEY WORDS

derivatization

REFERENCE

Toyo'oka,T.; Kuze,A. Determination of saccharides labelled with a fluorescent reagent, DBD-ProCZ, by liquid chromatography, *Biomed.Chromatogr.*, **1997**, *11*, 132–136.

SAMPLE

Matrix: tissue

Sample preparation: 10 g Meat + 52 mL EtOH, make up to 100 mL with water, stir magnetically for 10 min, filter (paper) about 45 mL. 20 mL Filtrate + 15 mL petroleum ether, shake vigorously for 1 min, let stand for 5 min, wash twice more with petroleum ether. Add aqueous layer to a Sep-Pak C18 SPE cartridge, evaporate the effluent to dryness under vacuum at 40°, reconstitute the residue in 2 mL water, add to ion-exchange columns, rinse flask four times with 4 mL water, add rinses to ion-exchange columns. Combine all column effluents, add 5 mL EtOH, evaporate to dryness under vacuum at 40°. Reconstitute the residue in 1 mL water, vortex for 45 s, let stand for 1 min, filter (0.45 μm Fluoropore), inject an aliquot. (Prepare ion-exchange columns as follows. Rinse resins several times with water until supernatants are colorless and pH is unchanged. Place AG 50W-X8 50-100 mesh cation exchange resin (hydrogen form) to a height of 38 mm in an 8 mm i.d. polypropylene column (column I). Place AG 3-X4A 20-50 mesh anion exchange resin (chloride form) to a height of 14 mm in an 8 mm i.d. polypropylene column. Wash with two volumes 500 mM NaOH at 1 mL/min and with water until chloride free (test with nitric acid/silver nitrate) (column II). Place column I above column II.)

HPLC VARIABLES
Column: 250 × 4 Bio-Sil Amino 5S (Bio-Rad)
Mobile phase: MeCN:water 78:22
Flow rate: 1
Detector: RI

CHROMATOGRAM
Retention time: 14.25

OTHER SUBSTANCES
Extracted: sucrose, maltose, lactose

KEY WORDS
meat; sausage; SPE

REFERENCE
Ali,M.S. Simultaneous determination of dextrose, sucrose, maltose, and lactose in sausage products by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1097–1100.

SAMPLE
Matrix: urine
Sample preparation: Mix acetone with urine so as to make a 63:47 acetone:urine mixture, centrifuge a 6 mL aliquot. Evaporate the acetone from the supernatant under a stream of helium at 35°, add 30 mg Dowex 50W-X8, add 30 mg Dowex 1-X8, agitate, centrifuge, inject a 1-10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.3 Hitachi 3013 N anion-exchange resin, phosphate form
Mobile phase: MeCN:water 83:17
Column temperature: 60
Flow rate: 1
Injection volume: 1-10
Detector: UV 530 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min, the mixture flowed through a 3 m × 0.5 mm i.d. coil of PTFE tubing at 85° and a 1 m × 0.5 mm i.d. coil of PTFE tubing at room temperature to the detector. (Reagent was 2 g/L blue tetrazolium in EtOH:water 50:50 containing 180 mM NaOH.)

CHROMATOGRAM
Retention time: 23
Limit of detection: 10 ng

OTHER SUBSTANCES
Extracted: arabinose, fructose, fucose, galactose, lactose, xylose, ribose

KEY WORDS
post-column reaction

REFERENCE
D'Amboise,M.; Hanai,T.; Noël,D. Liquid-chromatographic measurement of urinary monosaccharides, *Clin.Chem.*, **1980**, *26*, 1348–1350.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 10 μL 10% trichloroacetic acid in water + 50 μL 5% dansyl hydrazine in MeCN, heat at 65° for 20 min, cool in ice, add an equal volume of water, inject a 10-300 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:80 mM acetic acid 21:79
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em >470

CHROMATOGRAM

Retention time: 9
Limit of detection: 5-15 pmole

OTHER SUBSTANCES

Extracted: arabinose, cellobiose, 2-deoxyglucose, 2-deoxyribose, fructose, fucose, galactose, gentobiose, lactose, maltose, mannose, rhamnose, ribose, xylose

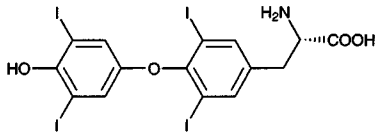
KEY WORDS

derivatization

REFERENCE

Mopper,K.; Johnson,L. Reversed-phase liquid chromatographic analysis of Dns-sugars. Optimization of derivatization and chromatographic procedures and applications to natural samples, *J.Chromatogr.*, **1983**, *256*, 27–38.

Dextrothyroxine



Molecular formula: $C_{15}H_{11}I_4NO_4$
Molecular weight: 776.87
CAS Registry No.: 51-49-0, 137-53-1 (monosodium salt), 7054-08-2 (monosodium salt hydrate)
Merck Index: 9555
Lednicer No.: 1 92

SAMPLE

Matrix: microsomal incubations
Sample preparation: Mix 1 mL microsomal incubation with 200 µL 5% trichloroacetic acid. Mix with 3 mL 500 mM pH 9.3 ammonium sulfate and add to a Sep-Pak C18 SPE cartridge. Wash with 20 mL 5 mM pH 9.3 ammonium sulfate, wash with 500 µL water. Elute with 3 mL MeCN:MeOH:20 mM pH 2.1 potassium phosphate buffer 40:38:22, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4 3 µm Hypersil ODS
Mobile phase: MeCN:MeOH:20 mM pH 1.8 potassium phosphate buffer containing 2.5 mM sodium dodecyl sulfate 40:10:50
Flow rate: 0.5 for 5 min, 1.2 for 15 min
Detector: UV 234

CHROMATOGRAM

Retention time: 18.3

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver; SPE

REFERENCE

Jemnitz,K.; Vereczkey,L. Ion-pair high-performance liquid chromatographic separation of two thyroxine glucuronides formed by rat liver microsomes, *J.Chromatogr.B*, **1996**, *681*, 385–389.

SAMPLE

Matrix: solutions
Sample preparation: Take up 1.5 mg dextrothyroxine in 200 µL 100 mM sodium bicarbonate and 400 µL reagent, stir in an ice bath for 30 min, evaporate to dryness below 30°, add 100 µL trifluoroacetic acid to the dry residue, let stand for 30 min at room temperature, add 2 mL 1 M sodium bicarbonate, centrifuge. Remove the precipitate and dissolve it in 600 µL MeOH:20 mM NaOH 50:50, inject a 15 µL aliquot. Reagent was 7 mg/mL BOC-L-Leu-SU (tert-butyloxy-L-leucine-N-hydroxysuccinimide ester) in MeOH, prepared immediately before use.)

HPLC VARIABLES

Column: 150 × 3.2 7 µm LiChrosorb RP-18
Mobile phase: MeOH:water 60:40 containing 0.05% methanesulfonic acid
Flow rate: 1
Injection volume: 15
Detector: UV 230

CHROMATOGRAM

Retention time: 11.5

Limit of detection: 0.05% of the L form

OTHER SUBSTANCES

Simultaneous: levothyroxine, impurities

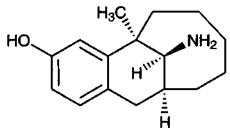
KEY WORDS

derivatization; chiral

REFERENCE

Lankmayr,E.P.; Budna,K.W.; Nachtmann,F. Separation of enantiomeric iodinated thyronines by liquid chromatography of diastereomers, *J.Chromatogr.*, **1980**, *198*, 471–479.

Dezocine



Molecular formula: $C_{16}H_{23}NO$
Molecular weight: 245.36
CAS Registry No.: 53648-55-8
Merck Index: 2998
Lednicer No.: 4 59

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum or plasma + 0.5 μg/mL IS in water + 1 mL 5 M ammonium hydroxide, vortex briefly, add 5 mL ethyl acetate, vortex for 5 min, centrifuge at 400 g for 15 min. Remove the organic layer and evaporate to dryness under vacuum at 40°, reconstitute in 200 μL mobile phase, inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 120 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:butanol:phosphoric acid 289:650:10:1
Flow rate: 0.8
Injection volume: 20-50
Detector: E, Environmental Sciences Associates Coulochem Model 5100A, Model 5010 analytical cell, detector 1 +0.58 μA, detector 2 + 0.84 μA, Model 5020 guard cell +0.90 μA

CHROMATOGRAM

Retention time: 4.1
Internal standard: 11-amino-5,6,7,8,9,10-hexahydro-5-phenyl-5,9-methanobenzocyclooctene-3-ol (Wy 17288) (5.4)
Limit of detection: 1-2 ng/mL

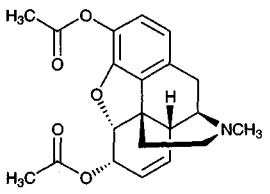
KEY WORDS

serum; plasma; pharmacokinetics

REFERENCE

Locniskar,A.; Greenblatt,D.J. Determination of ciramadol and dezocine, two new analgesics, by high-performance liquid chromatography using electrochemical detection, *J.Chromatogr.*, **1986**, *374*, 215–220.

Diamorphine



Molecular formula: $C_{21}H_{23}NO_5$
Molecular weight: 369.42
CAS Registry No.: 561-27-3, 1502-95-0 (hydrochloride)
Merck Index: 3012
Lednicer No.: 1 288

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg ethyl SPE cartridge (J.T.Baker) with 2 mL MeOH, 1 mL water, and 2 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer. Mix 1 mL serum with 200 μL 1 μg/mL IS in water. Add to the SPE cartridge, wash with 1 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 μm Supelcosil LC-Si (Supelco)
Mobile phase: MeCN:MeOH:water:formic acid 5.2:59.8:34.65:0.35
Flow rate: 0.23
Injection volume: 5
Detector: MS, API I MS single quadrupole, ionspray, capillary tip 5000 V, interface plate 650 V, source 60°, positive ion mode, orifice 70 V, SIM, m/z 370

CHROMATOGRAM

Retention time: 20.52
Internal standard: nalorphine (15.4)
Limit of quantitation: 500 pg/mL

OTHER SUBSTANCES

Extracted: metabolites, codeine, morphine

KEY WORDS

serum; pharmacokinetics; SPE; mouse

REFERENCE

Zuccaro,P.; Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; D'Ascenzo,G. Simultaneous determination of heroin, 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography-atmospheric pressure ionspray-mass spectrometry, *J.Anal.Toxicol.*, **1997**, *21*, 268–277.

SAMPLE

Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8

Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 18
Limit of detection: 0.40 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS
whole blood

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 11.152

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 23.1

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (Alltech)
Mobile phase: MeCN:water 45:55 containing 10 μL triethylamine per 100 mL
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.1

OTHER SUBSTANCES
Also analyzed: codeine, fentanyl, meperidine, morphine

REFERENCE
Lichtman,A.H.; Meng,Y.; Martin,B.R. Inhalation exposure to volatilized opioids produces antinociception in mice, *J.Pharmacol.Exp.Ther.*, **1996**, *279*, 69–76.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W

Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.19

OTHER SUBSTANCES
Simultaneous: thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: epinephrine, pipradol, phenylpropanolamine, fencamfamin, hydroxypethidine, normethadone, meperidine, dipipanone, pentazocine, acetylcodeine, monoacetylmorphine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine,

estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Condition a 300 mg Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL water. 5 mL Urine + 1 mL concentrated HCl, vortex, heat at 120° for 30 min, cool, adjust pH to between 7.0 and 8.0 with 10 M KOH. 5 mL Urine or hydrolysed urine + nalorphine, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL pH 4 acetate buffer, wash with 2 mL MeOH, elute with 2 mL dichloromethane:isopropanol 80:20 containing 2% ammonia. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 0.5-1 mL pentane:dichloromethane 90:10. (Use unhydrolysed urine to determine diamorphine and unconjugated compounds.)

HPLC VARIABLES

Column: 200 × 2 3 μm Hypersil
Mobile phase: Pentane:dichloromethane:MeOH containing 0.5% diethylamine 65:29.8:5.2
Flow rate: 0.4
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 4
Internal standard: nalorphine (5)

Limit of detection: <20 ng/mL

OTHER SUBSTANCES

Extracted: codeine, 6-monoacetylmorphine, pholcodine, dihydrocodeine, morphine
Simultaneous: diphenhydramine, ephedrine, hydrocodone
Noninterfering: aspirin, caffeine, chlordiazepoxide, dextropropoxyphene, diazepam, lignocaine, naloxone, norcodeine, normorphine, papaverine, procaine, quinine, theobromine, theophylline

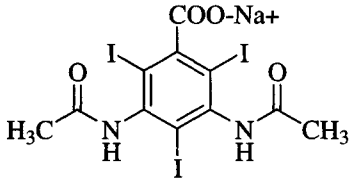
KEY WORDS

normal phase; SPE

REFERENCE

Low,A.S.; Taylor,R.B. Analysis of common opiates and heroin metabolites in urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 225–233.

Diatrizoate sodium



Molecular formula: $C_{11}H_8I_3N_2NaO_4$
Molecular weight: 635.90
CAS Registry No.: 737-31-5, 117-96-4 (free acid), 50978-11-5 (free acid, dihydrate), 131-49-7 (diatrizoate meglumine)
Merck Index: 3040

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 50 μL Plasma + 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. Tissue. Weigh 2 testes, add 5 mL buffer, homogenize (Kinematica type PT 10/35, setting 7.5) for 1 min, vortex, centrifuge at 1000 g at 0° for 10 min. Remove a 50 μL aliquot of the supernatant and add it to 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. (Buffer was 5 mM metaphosphoric acid and 5 mM disodium EDTA.)

HPLC VARIABLES

Guard column: C18 Bondapak guard column
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 2.5:97.5–5:95 (Buffer was 100 mM NaH_2PO_4 and 0.2 mM Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 9.5
Limit of detection: 710 ng/mL

OTHER SUBSTANCES

Also analyzed: iohexol, iopamidol

KEY WORDS

plasma; mouse; testes

REFERENCE

Harapanhalli,R.S.; Yaghmai,V.; Patel,Y.D.; Baker,S.R.; Rao,D.V. Assay of radiographic contrast agents in mice plasma and testes by high-performance liquid chromatography, *Anal.Chem.*, **1993**, *65*, 606–612.

SAMPLE

Matrix: formulations
Sample preparation: Dilute 1 mL to 1 L with water, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 5 μm Resolve C18 (Waters)
Mobile phase: Water:85% phosphoric acid 99.75:0.25
Flow rate: 1
Injection volume: 10
Detector: UV 238

CHROMATOGRAM

Retention time: 2
Limit of quantitation: 5 ppm

OTHER SUBSTANCES

Simultaneous: impurities, degradation products

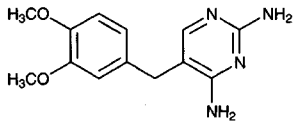
KEY WORDS

injections

REFERENCE

Farag,S.A. Liquid chromatographic assay of diatrizoic acid and its diiodo degradation products in radiopaque solutions, *J.AOAC Int.*, **1995**, *78*, 328–333.

Diaveridine



Molecular formula: $C_{13}H_{16}N_4O_2$
Molecular weight: 260.30
CAS Registry No.: 5355-16-8
Merck Index: 3041

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 7.022

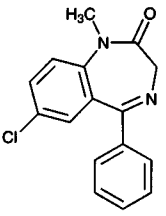
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Diazepam



Molecular formula: $C_{16}H_{13}ClN_2O$
Molecular weight: 284.74
CAS Registry No.: 439-14-5
Merck Index: 3042
Lednicer No.: 1 365; 2 395

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine

Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 10 μL 1 mg/mL prazepam and 1 mL pH 7.4 phosphate buffer, vortex briefly, add 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot of the solution.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II ODS
Mobile phase: MeCN:MeOH:10 mM phosphoric acid:10 mM Na_2HPO_4 40:20:36:4
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 8.5
Internal standard: prazepam (14.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, nitrazepam, oxazepam, temazepam

KEY WORDS

liver; lung; muscle; urine; pericardial fluid

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood

Sample preparation: Add 40 μL 2% NaOH, and 3.5 mL cyclohexane:diethyl ether 31:69 to 1 mL plasma. Extract on a rotary mixer at 4° for 10 min, centrifuge at 4° at 2000 g for 10 min. Remove a 3.3 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°. Dissolve the residue in 300 μL MeCN:water 5:95, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 5 × 0.8 μ-Precolumn cartridge C18 (LC Packings)
Column: 150 × 0.8 3 μm Hypersil C18 BDS

Mobile phase: Gradient. MeCN:10 mM pH 7.0 sodium phosphate buffer 35:65 for 16 min, to 60:40 over 1 min, maintain at 60:40.
Flow rate: 0.016
Injection volume: 20
Detector: UV 240 for 17.6 min then UV 300

CHROMATOGRAM
Retention time: 19.5
Internal standard: diazepam

OTHER SUBSTANCES
Extracted: midazolam

KEY WORDS
capillary HPLC; plasma; diazepam is IS

REFERENCE
Eeckhoudt,S.L.; Desager,J.-P.; Horsmans,Y.; De Winne,A.J.; Verbeeck,R.K. Sensitive assay for midazolam and its metabolite 1'-hydroxymidazolam in human plasma by capillary high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *710*, 165–171.

SAMPLE
Matrix: blood
Sample preparation: Vortex 500 μL plasma, 5 mL ether, and 3 mL 100 mM HCl, for 1 min, centrifuge at 3000 rpm for 3 min. Remove the aqueous phase, add 7 mL chloroform (Caution! Chloroform is a carcinogen!) and 500 μL 1 M NaOH. Shake the mixture for 2 min, remove the chloroform phase, evaporate it to dryness under vacuum at 45°. Reconstitute the residue with 500 μL mobile phase, vortex for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:buffer 55:45 (Buffer was water containing 200 mM ammonium acetate, adjusted to pH 7.1–7.3 with acetic acid.)
Column temperature: 38
Flow rate: 1.5
Injection volume: 20
Detector: UV 249

CHROMATOGRAM
Retention time: 5.1
Internal standard: diazepam

OTHER SUBSTANCES
Extracted: haloperidol

KEY WORDS
plasma; diazepam is IS

REFERENCE
El-Sayed,Y.M.; Khidr,S.H.; Niazy,E.M. High-performance liquid chromatographic assay for the determination of haloperidol in plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 125–134.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate

the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 Mm pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 29.8 (A), 77.5 (B)
Internal standard: diazepam

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum; diazepam is IS

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 μL 2 μg/mL IS in MeOH, add 2 mL water and 2 mL MeCN, vortex gently, set aside for 3 min, centrifuge at 2200 g for 20 min. Separate the clear supernatant, add 500 μL 200 mM NaOH and extract with 6 mL n-hexane by vortexing for 2 min. Centrifuge at 2200 g for 15 min. Evaporate 5 mL organic phase to dryness. Reconstitute the residue in 120 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 5 μm AGP bonded silica (ChromTech, Hagersten)
Column: 150 × 4 5 μm AGP bonded silica (ChromTech, Hagersten)
Mobile phase: 8 mM sodium dihydrogen phosphate + 0.1 M NaCl:n-propanol:diethylamine 95.4:4:0.6, adjust to pH 7.05 with 50% phosphoric acid
Flow rate: 0.9
Injection volume: 100
Detector: UV 214

CHROMATOGRAM

Retention time: 19.21

OTHER SUBSTANCES

Simultaneous: lidocaine, bupivacaine

KEY WORDS

plasma; pharmacokinetics; diazepam is IS

REFERENCE

Abraham,I.; Fawcett,J.P.; Kennedy,J.; Kumar,A.; Ledger,R. Simultaneous analysis of lignocaine and bupivacaine enantiomers in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *703*, 203–208.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, urine. 500 μL Serum or urine + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl, centrifuge at 15 000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)

Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40

Flow rate: 0.6

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 4.4 (A)

Internal standard: diazepam

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver; diazepam is IS

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.327

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: Nova-Pak C18
Mobile phase: MeOH:buffer 85:15 (Buffer was 90.7 mL 66.7 mM Na_2HPO_4 and 9.3 mL 66.7 mM KH_2PO_4 made up to 1 L with water, pH 7.6.)
Flow rate: 5 (sic)
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 6.78
Limit of detection: 50 nM

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, flurazepam, nitrazepam

KEY WORDS
comparison with capillary electrophoresis; capillary GC; and polarography

REFERENCE
McGrath,G.; McClean,S.; O'Kane,E.; Smyth,W.F.; Tagliaro,F. Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay, *J.Chromatogr.A*, **1996**, *735*, 237–247.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb ODS-2
Mobile phase: MeCN:MeOH:water 5:45:50
Flow rate: 2
Detector: UV 228

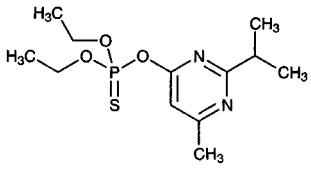
CHROMATOGRAM

Retention time: 12

REFERENCE

Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

Diazinon



Molecular formula: $C_{12}H_{21}N_2O_3PS$
Molecular weight: 304.35
CAS Registry No.: 333-41-5
Merck Index: 3043

SAMPLE

Matrix: blood
Sample preparation: 1.5 mL Serum + 2 mL 200 mM pH 7.0 phosphate buffer, add to an Extrelut No. 3 SPE column, let stand for 10 min, elute with 15 mL n-hexane:diethyl ether 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 150 μL MeOH:water 70:30, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeOH:water from 70:30 to 90:10.
Flow rate: 1
Injection volume: 200
Detector: MS, Hitachi Model M-2000, APCI non-equilibrium interface, vaporizer 250°, nebulizer 400°, ionization needle electrode current 5 μA, drift voltage 230 V, vacuum 0.0001 Pa, ion-source slit 500 μm, collector slit 400 μm, accelerated electrical potential 4 kV, secondary electronic step-up tube potential 1.3 kV, positive-ion mode

CHROMATOGRAM

Retention time: 11.4
Limit of detection: 5 ng

OTHER SUBSTANCES

Extracted: dichlorvos, dimethoate, dimethylvinphos, ediphenphos, fenthion, IBP, isoxathon, malathion, phenthoate, propaphos, pyridafenthion

KEY WORDS

serum; SPE; m/z 305

REFERENCE

Kawasaki,S.; Ueda,H.; Itoh,H.; Tadano,J. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1992**, *595*, 193–202.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 25.763

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Mix 1 g sample with 10 mL THF, after total dissolution add 35 mL THF, filter the polyvinyl chloride precipitate. Repeat this operation, then add 4 mL 6 mg/mL benzophenone in dioxane and dilute to 100 mL with EtOH. Inject a 5 μL aliquot. (Caution! Dioxane is a carcinogen!)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Kromasil 110 ODS C18
Mobile phase: MeCN:water 85:15
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 5.77
Internal standard: benzophenone (4.23)

OTHER SUBSTANCES
Simultaneous: dibutyl phthalate

KEY WORDS
polymer matrix

REFERENCE
Delhom,N.; Balanant,Y.; Ader,J.C.; Lattes,A. High performance liquid chromatographic determination of diazinon in polymeric matrix, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1735–1743.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Inject a 10 μL aliquot directly.

HPLC VARIABLES
Column: 120 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:water 80:20
Flow rate: 1
Injection volume: 10

Detector: UV 220

CHROMATOGRAM
Retention time: 1

OTHER SUBSTANCES
Simultaneous: azinphos methyl, parathion methyl

REFERENCE
Farran,A.; De Pablo,J.; Barceló,D. Identification of organophosphorus insecticides and their hydrolysis products by liquid chromatography in combination with UV and thermospray-mass spectrometric detection, *J.Chromatogr.*, **1988**, *455*, 163–172.

SAMPLE
Matrix: solutions
Sample preparation: Make up a 1 mg/mL solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Partisil
Mobile phase: Cyclohexane:dioxane 97:3 (85:15 for some more polar impurities)
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
normal phase

REFERENCE
Nichol,A.W.; Elsbury,S.; Elder,G.H.; Jackson,A.H.; Rao,K.R.N. Separation of impurities in diazinon preparations and their effect on porphyrin biosynthesis in tissue culture, *Biochem.Pharmacol.*, **1982**, *31*, 1033–1038.

SAMPLE
Matrix: solutions
Sample preparation: Equilibrate column A with 10 mL MeCN and 10 mL water (pH 7). Pump 200 mL drinking water through column A at 3 mL/min, back flush contents of column A onto column B with the mobile phase and start the gradient.

HPLC VARIABLES
Column: A 10 × 2.1 5 μm RP-18 octadecylsilica (E. Merck); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:water from 40:60 to 60:40 over 15 min
Injection volume: 200000
Detector: UV 254

CHROMATOGRAM
Retention time: 19.75
Limit of detection: 0.06 ng/mL

OTHER SUBSTANCES
Extracted: azinphos-methyl, carbaryl, parathion-methyl, azinphos-ethyl, fenitrothion, parathion, phosmet

KEY WORDS
drinking water; column-switching

REFERENCE
Driss,M.R.; Hennion,M.-C.; Bouguerra,M.L. Determination of carbaryl and some organophosphorus pesticides in drinking water using on-line liquid chromatographic preconcentration techniques, *J.Chromatogr.*, **1993**, *639*, 352–358.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 C18
Mobile phase: Gradient. Isopropanol:20 mM pH 2.5 sodium phosphate buffer containing 20 mM sodium dodecyl sulfate from 10:90 to 50:50 over 15 min
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: aldicarb, chloroxuron, diuron, fluometuron, linuron, metobromuron, monuron, neburon, parathion, prometryne, propazine, siduron, terbacil
Interfering: prometon

REFERENCE
Zhang,Y.; El Rassi,Z. High performance micellar liquid chromatography with silica microparticles having surface-bound cationic surfactant moieties. I. Comparison with octadecylsilica and applications to the separation of dabsyl amino acids, herbicides, and catecholamines, *J.Liq.Chromatogr.*, **1995**, *18*, 3373–3396.

SAMPLE
Matrix: solutions
Sample preparation: Condition a 10 × 2 SPE column packed with 40 μm octadecylsilica (Spark Holland) with 10 mL MeCN, 10 mL MeOH, and 10 mL water at 2 mL/min. Add nitric acid to a final concentration of 0.5% to water sample, filter (0.45 μm), add a 150 mL aliquot to the SPE column at 3 mL/min, wash with 3 mL distilled water, elute the contents of the SPE column on to the analytical column with the mobile phase.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 60 RP-8 endcapped C8 (Merck)
Mobile phase: Gradient. A was MeCN:MeOH 80:20. B was water. A:B from 10:90 to 40:60 over 10 min, maintain at 40:60 for 5 min, to 90:10 over 33 min, return to initial conditions over 5 min.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 39.8

OTHER SUBSTANCES
Simultaneous: azinphos-ethyl, azinphos-methyl, chlorfenvinphos, dichlorvos, fenitrothion, malathion, mevinphos, parathion-ethyl, parathion-methyl
Interfering: fenthion

KEY WORDS
groundwater; wastewater; SPE

REFERENCE
Lacorte,S.; Barceló,D. Improvements in the determination of organophosphorus pesticides in ground- and wastewater samples from interlaboratory studies by automated on-line liquid-solid extraction followed by liquid chromatography-diode array detection, *J.Chromatogr.A*, **1996**, *725*, 85–92.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 mL river water on to column A at 5 mL/min and let the effluent flow to waste, backflush the contents of column A on to column B and start the gradient, monitor the effluent from column B. At the end of each run backflush column A with 5 mL water, with 30 mL 100 mM pH 2 sodium citrate buffer, with 10 mL water, with 5 mL MeCN, and with 10 mL hexane:dichloromethane 50:50. Wash new column A with 10 mL water.

HPLC VARIABLES
Column: A 20 × 3 10 μm PRP-1 (Hamilton); B 30 × 4.6 10 μm RP-18 (Brownlee) + 250 × 4.6 5 μm LiChrospher C18
Mobile phase: Gradient. MeCN:water from 5:95 to 90:10 over 1 h, maintain at 90:10 for 4 min, return to initial conditions (?) over 1 min, re-equilibrate for 10 min.
Flow rate: 1 for 64 min, to 1.5 over 1 min, maintain at 1.5 for 5 min, to 1 over 5 min
Injection volume: 100000
Detector: UV 210

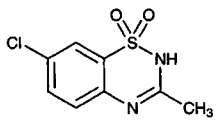
CHROMATOGRAM
Retention time: 47.5
Limit of detection: 50 ng/L

OTHER SUBSTANCES
Simultaneous: alachlor, aldicarb, aldicarb oxime, atrazine, carbofuran, chlorobenzilate, chlorothalonil, chlorpyrifos methyl, chlortoluron, cypermethrin, p,p'-DDE, DDT, deltamethrin, diclofop methyl, dimethoate, diuron, ethofumesate, fenitrothion, fenvalerate, fluazifop butyl, fluometuron, linuron, metalaxyl, metamitron, methomyl, metobromuron, metolachlor, molinate, oxamyl, paraoxon, paraoxon methyl, parathion methyl, pendimethalin, permethrin, phenmediphan, pirimphos, pirimphos methyl, prometryne, propanil, propiconazole, simazine, terbuthylazine, trifluraline
Interfering: parathion

KEY WORDS
river water; column-switching

REFERENCE
Papadopoulou-Mourkidou,E.; Patsias,J. Development of a semi-automated high-performance liquid chromatographic-diode array detection system for screening pesticides at trace levels in aquatic systems of the Axios River basin, *J.Chromatogr.A*, **1996**, *726*, 99–113.

Diazoxide



Molecular formula: $C_8H_7ClN_2O_2S$
Molecular weight: 230.67
CAS Registry No.: 364-98-7
Merck Index: 3051

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 265

CHROMATOGRAM

Retention time: 3.13
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, saliva

Sample preparation: Serum. 200 μL Serum + 100 μL buffer + 50 μL 20% perchloric acid, vortex, centrifuge at 2000 g for 5 min, inject a 250 μL aliquot of the supernatant onto column A, then inject 700 μL buffer onto column A, elute the contents of column A onto column B with mobile phase for 1 min, remove column A from circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with 2 mL MeOH:buffer 50:50 then forward flush with 1 mL buffer. Saliva. Centrifuge saliva at 3000 g for 4 min. 100 μL Supernatant + 150 μL water + 100 μL buffer, vortex, inject a 250 μL aliquot onto column A, then inject 700 μL buffer onto column A, elute the contents of column A onto column B with mobile phase for 1 min, remove column A from circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with 2 mL MeOH:buffer 50:50 then forward flush with 1 mL buffer. (Buffer was 50 mM pH 4.6 $(NH_4)H_2PO_4$.)

HPLC VARIABLES

Column: A 30 mm long 30-40 μm C18; B 10 × 4.6 5 μm Spherisorb S5 ODS-1 C18 end-capped + 250 × 4.6 5 μm Spherisorb S5 ODS-1 C18 end-capped

Mobile phase: MeCN:buffer 12:88 (Buffer was 50 mM pH 4.6 $(NH_4)H_2PO_4$.)

Column temperature: 40

Flow rate: 2

Injection volume: 250

Detector: UV 295

CHROMATOGRAM

Retention time: 8.5

Internal standard: diazoxide (8.5)

OTHER SUBSTANCES

Extracted: dapsone

KEY WORDS

serum; diazoxide is IS; column-switching

REFERENCE

Moncrieff,J. Determination of dapsone in serum and saliva using reversed-phase high-performance liquid chromatography with ultraviolet or electrochemical detection, *J.Chromatogr.B*, **1994**, *654*, 103–110.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 6-10 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM

Retention time: 3.42
Internal standard: methsuximide (5.30)

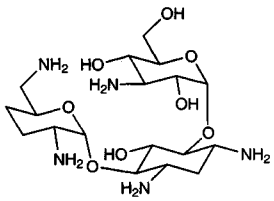
OTHER SUBSTANCES

Simultaneous: acetaminophen, N-acetylcysteine, N-acetylprocainamide, amobarbital, ampicillin, aspirin, barbital, butabarbital, butalbital, caffeine, carbamazepine, chloramphenicol, chlorpropamide, cimetidine, codeine, cyheptamide, diphylline, disopyramide, ethchlorvynol, ethosuximide, gentisic acid, glutethimide, heptabarbital, hexobarbital, indomethacin, ketoprofen, mefenamic acid, mephenytoin, mephobarbital, methaqualone, methyl salicylate, morphine, naproxen, oxphenylbutazone, pentobarbital, phenacetin, phensuximide, phenylbutazone, phenytoin, primidone, procainamide, salicylamide, salicylic acid, secobarbital, sulfamethoxazole, sulindac, theophylline, thiopental, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: acetanilide, diflunisal, ibuprofen, methyprylon, nirvanol, phenobarbital

REFERENCE

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

Dibekacin



Molecular formula: $C_{18}H_{37}N_5O_8$
Molecular weight: 451.52
CAS Registry No.: 34493-98-6, 58580-55-5 (sulfate)
Merck Index: 3052

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 2 mL EtOH. Add 7 mL dichloromethane and 1 mL water, mix, centrifuge. Inject an aliquot of the aqueous supernatant onto column A, elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, elute column B with mobile phase B. Mix the effluent from column B with o-phthalaldehyde at 0.2 mL/min and monitor.

HPLC VARIABLES

Column: A 3.9 × 4.0 10 μm Guard Pak Cyano (Waters); B 150 × 4.6 5 μm Shandon Hypersil C18
Mobile phase: A 17 mM acetic acid containing 10 mM hexanesulfonic acid; B 17 mM acetic acid containing 10 mM hexanesulfonic acid, 100 mM sodium acetate, and 3.53 M MeOH
Detector: F ex 338 em 450 (cut-off filter) following post-column reaction. The column effluent mixed with o-phthalaldehyde pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM

Retention time: 9.5
Internal standard: dibekacin
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: isepamicin
Simultaneous: aspirin, caffeine, chlorpheniramine, gentamicin, neomycin, netilmicin, sisomicin

KEY WORDS

plasma; post-column reaction; column-switching; dibekacin is IS

REFERENCE

Lin,C.; Veals,J.; Korduba,C.; Hilbert,M.J.; Nomeir,A. Analysis of isepamicin in human plasma by radioimmunoassay, microbiologic assay, and high-performance liquid chromatography, *Ther.Drug Monit.*, **1997**, *19*, 675–681.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.053

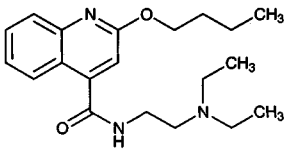
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Dibucaine



Molecular formula: $C_{20}H_{29}N_3O_2$
Molecular weight: 343.47
CAS Registry No.: 85-79-0, 61-12-1 (HCl)
Merck Index: 3081
Lednicer No.: 1 15

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL dexamethasone + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 25:20:55
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Internal standard: dexamethasone

KEY WORDS

plasma; rat

REFERENCE

Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE

Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 10 μL 100 μg/mL etidocaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL 10 mM HCl, add 3 mL diethyl ether, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM

Retention time: 18
Internal standard: etidocaine (14)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine, prilocaine, bupivacaine
Also analyzed: procaine, butacaine, tetracaine, p-aminobenzoic acid, articaine, o-toluidine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.75

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 4 mL urine to 11 with concentrated ammonium hydroxide, add 200 μL 20 μg/mL IS, add 5 mL ethyl acetate, shake vigorously for 1 min, centrifuge. Remove the organic layer and add it to 2 mL 500 mM HCl, shake. Remove the aqueous layer and make it alkaline with concentrated ammonium hydroxide, extract with 5 mL dichloromethane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5 C18 (Nakarai Chemical Co.)
Mobile phase: MeOH:water 60:40 containing 30 mM triethylamine, pH adjusted to 7.5 with acetic acid
Flow rate: 1
Injection volume: 10
Detector: F ex 330 em 440

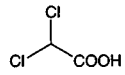
CHROMATOGRAM
Retention time: 27
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE

Igarashi,K.; Kasuya,F.; Fukui,M.; Nanjyou,H. Determination of dibucaine and its metabolites in human urine by high-performance liquid chromatography with fluorescence detector, *Chem.Pharm.Bull.(Tokyo)*, **1987**, *35*, 3033–3036.

Dichloroacetic acid



Molecular formula: $C_2H_2Cl_2O_2$
Molecular weight: 128.94
CAS Registry No.: 79-43-6
Merck Index: 3100

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.2 5 μm Spherisorb C18
Mobile phase: 150 mM ammonium sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 3.92

OTHER SUBSTANCES
Simultaneous: acetic acid, chloroacetic acid, trichloroacetic acid

REFERENCE
Husain,S.; Narsimha,R.; Alvi,S.N.; Rao,R.N. Monitoring the levels of chloroacetic acids in the industrial environment by high performance liquid chromatography, *J.High Res.Chromatogr.*, **1993**, *16*, 381–383.

SAMPLE
Matrix: urine
Sample preparation: Hydrolyze with 1000 U/mL β-glucuronidase (Type VII, Sigma) in 100 mM pH 7.0 phosphate buffer at 37° for 2 h, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 200 × 8 30-35 μm Aminex 50W-X4 (Bio-Rad)
Mobile phase: 10 mM HCl
Column temperature: 65
Flow rate: 1
Injection volume: 100
Detector: UV 210

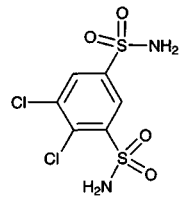
CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: trichloroacetic acid, chloroacetic acid, trichloroethanol

KEY WORDS
mouse

REFERENCE
Green,T.; Prout,M.S. Species differences in response to trichloroethylene. II. Biotransformation in rats and mice, *Toxicol.Appl.Pharmacol.*, **1985**, *79*, 401–411.

Dichlorphenamide



Molecular formula: $C_6H_6Cl_2N_2O_4S_2$
Molecular weight: 305.16
CAS Registry No.: 120-97-8
Merck Index: 3127
Lednicer No.: 1 133

SAMPLE

Matrix: urine
Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 µL 50 µg/mL β-hydroxyethyltheophylline in MeOH, inject 5 µL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 9.37 (A), 10.3 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 µL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 14.1

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, cvhlorthalidone, clopamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

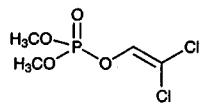
KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

Dichlorvos



Molecular formula: $C_4H_7Cl_2O_4P$
Molecular weight: 220.98
CAS Registry No.: 62-73-7
Merck Index: 3129

SAMPLE

Matrix: blood
Sample preparation: 1.5 mL Serum + 2 mL 200 mM pH 7.0 phosphate buffer, add to an Extrelut No. 3 SPE column, let stand for 10 min, elute with 15 mL n-hexane:diethyl ether 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 150 µL MeOH:water 70:30, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: Gradient. MeOH:water from 70:30 to 90:10.
Flow rate: 1
Injection volume: 200
Detector: MS, Hitachi Model M-2000, APCI non-equilibrium interface, vaporizer 250°, nebulizer 400°, ionization needle electrode current 5 µA, drift voltage 230 V, vacuum 0.0001 Pa, ion-source slit 500 µm, collector slit 400 µm, accelerated electrical potential 4 kV, secondary electronic step-up tube potential 1.3 kV, positive-ion mode

CHROMATOGRAM

Retention time: 4.4
Limit of detection: 2 ng

OTHER SUBSTANCES

Extracted: diazinon, dimethoate, dimethylvinphos, ediphenphos, fenthion, IBP, isoxathon, malathion, phenthoate, propaphos, pyridafenthion

KEY WORDS

serum; SPE; m/z 221

REFERENCE

Kawasaki,S.; Ueda,H.; Itoh,H.; Tadano,J. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1992**, *595*, 193–202.

SAMPLE

Matrix: blood
Sample preparation: 400 µL Plasma + 80 µL 6 M HCl, vortex for 1 min, centrifuge at 0-5° at 39000 g for 30 min. Freeze the supernatant, thaw, centrifuge at 0-5° at 11000 g for 5 min, filter (0.2 µm, Schleicher and Schuell RC58) while centrifuging at 0-5° at 3000 g for 15 min, inject a 200 µL aliquot of the filtrate. (Trichlorfon may degrade to dichlorvos during sample preparation unless whole blood is immediately acidified with phosphoric acid (J.Chromatogr. 1993,612, 336).)

HPLC VARIABLES

Guard column: 37-50 µm C18/Corasil
Column: 200 × 3.9 10 µm C18 (Waters)
Mobile phase: MeOH:water 30:70 containing 1 mM octanesulfonic acid, pH 3.0
Flow rate: 1.5
Injection volume: 200

Detector: UV 210

CHROMATOGRAM
Retention time: 12.6
Limit of detection: 40 ng/mL

KEY WORDS
plasma

REFERENCE
Unni,L.K.; Hannant,M.E.; Becker,R.E. High-performance liquid chromatographic method using ultraviolet detection for measuring metrifonate and dichlorvos levels in human plasma, *J.Chromatogr.*, **1992**, *573*, 99–103.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize tissue with an equal volume of water, treat with a saturated solution of calcium chloride, let stand overnight, filter. Extract filtrate, blood, or other body fluid with an equal volume of ether. Adjust pH of aqueous layer to 2 with 2 M HCl, extract with an equal volume of ether. Combine the ether layers, evaporate to dryness, recxonstitute in a suitable solvent, inject an aliquot. (Note that dichlorvos may be volatilized under these conditions. A hexane solution of dichlorvos was volatilized under a stream of nitrogen and all the dichlorvos disappeared (J.Chromatogr. 1992, 573, 99).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax cyano
Mobile phase: Iso-octane:ethyl acetate 80:20
Flow rate: 1
Injection volume: 20
Detector: RI

CHROMATOGRAM
Retention time: 6.25
Limit of detection: 100 ng

OTHER SUBSTANCES
Extracted: carbaryl, monocrotophos, quinalphos, malathion, phosphomidon, baygon
Interfering: methyl parathion

KEY WORDS
liver; lung

REFERENCE
Sharma,V.K.; Jadhav,R.K.; Rao,G.J.; Saraf,A.K.; Chandra,H. High performance liquid chromatographic method for the analysis of organophosphorus and carbamate pesticides, *Forensic Sci.Int.*, **1990**, *48*, 21–25.

SAMPLE
Matrix: solutions
Sample preparation: Condition a 10 × 2 SPE column packed with 40 μm octadecylsilica (Spark Holland) with 10 mL MeCN, 10 mL MeOH, and 10 mL water at 2 mL/min. Add nitric acid to a final concentration of 0.5% to water sample, filter (0.45 μm), add a 150 mL aliquot to the SPE column at 3 mL/min, wash with 3 mL distilled water, elute the contents of the SPE column on to the analytical column with the mobile phase.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 60 RP-8 endcapped C8 (Merck)

Mobile phase: Gradient. A was MeCN:MeOH 80:20. B was water. A:B from 10:90 to 40:60 over 10 min, maintain at 40:60 for 5 min, to 90:10 over 33 min, return to initial conditions over 5 min.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 19.1
Limit of detection: 99 ng/L

OTHER SUBSTANCES

Simultaneous: azinphos-ethyl, azinphos-methyl, chlorfenvinphos, diazinon, fenitrothion, fenthion, malathion, mevinphos, parathion-ethyl, parathion-methyl

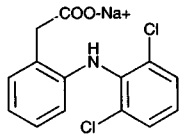
KEY WORDS

groundwater; wastewater; SPE

REFERENCE

Lacorte,S.; Barceló,D. Improvements in the determination of organophosphorus pesticides in ground- and wastewater samples from interlaboratory studies by automated on-line liquid-solid extraction followed by liquid chromatography-diode array detection, *J.Chromatogr.A*, **1996**, *725*, 85–92.

Diclofenac sodium



Molecular formula: $C_{14}H_{10}Cl_2NNaO_2$
Molecular weight: 318.13
CAS Registry No.: 15307-79-6
Merck Index: 3132
Lednicer No.: 2 70

SAMPLE

Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 400 ng/mL (+)-naproxen, mix mechanically for 90 s, centrifuge at 3000 g for 20 min. Remove supernatant and dry it under nitrogen at room temperature. Dissolve residue in 50 μL mobile phase by swirl mixing for 1 min, centrifuge at 3000 g for 20 s. For concentrations of < 20 ng/mL, reduce volume to 20-30 μL under nitrogen.

HPLC VARIABLES

Column: 150 × 4.5 5 μm Ultrasphere octyl
Mobile phase: 505 mL MeCN containing 0.65 mL triethylamine + 495 mL 1.65% glacial acetic acid, apparent pH 4.35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7.14
Internal standard: naproxen (3.89)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Extracted: flurbiprofen
Simultaneous: bacitracin, cortisone, diazepam, fluorometholone, hydrocortisone, imipramine, indomethacin, ketoprofen, ketorolac, levobunolol, meclofenamic acid, metipranolol, neomycin, prednisolone, proraracaine, propranolol, salicylic acid, sulfacetamide, suprofen
Noninterfering: acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide.

KEY WORDS

rabbit; human

REFERENCE

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 50 μL MeCN, mix, centrifuge at 12000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 3.2 5 μm NewGuard ODS
Column: 150 × 4 5 μm Nucleosil C18

Mobile phase: MeCN:buffer 32:68 (Buffer was 40 mL 1 M NaH_2PO_4 and 40 mL 500 mM Na_2HPO_4 made up to 680 mL with water, pH 6.6.)
Flow rate: 0.7
Injection volume: 50
Detector: F ex 288 em 360 following post-column reaction. The column effluent flowed through a 1.3 m × 1 mm ID PTFE tube irradiated by a UV 254 lamp (Philips TUV 6W, TYP 103314) to the detector.

CHROMATOGRAM
Retention time: 10
Limit of detection: 6 ng/mL

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; human; rat

REFERENCE
Wiese,B.; Hermansson,J. Bioanalysis of diclofenac as its fluorescent carbazole acetic acid derivative by a post-column photoderivatization high performance liquid chromatographic method, *J.Chromatogr.*, **1991**, *567*, 175–183.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 1.5 μg/mL IS in water + 4 mL 2 M phosphoric acid + 6 mL hexane:isopropanol 9:1, shake at 150 oscillations/min for 15 min, centrifuge at 1500 g for 10 min. Remove organic layer and evaporate it to dryness at 37° under a gentle stream of nitrogen. Reconstitute in 250 μL mobile phase, inject 100 μL aliquot onto column A, after 2 min switch eluent from column A onto column B, after another 2 min switch column A out of circuit and continue to flush it to waste with mobile phase. Monitor eluent from column B.

HPLC VARIABLES
Column: A 35 × 4.6 10 μm Nucleosil C18; B 150 × 4.6 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:22 mM pH 7.1 sodium acetate 23:25:52 (both columns)
Flow rate: 1.5
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 6.4
Internal standard: CGP-4287 (7.6)
Limit of detection: 2.5 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Miller,R.B. High-performance liquid chromatographic determination of diclofenac in human plasma using automated column switching, *J.Chromatogr.*, **1993**, *616*, 283–290.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 10 μL 50 μg/mL mefenamic acid + 50 μL 85% phosphoric acid, vortex 10 sec, add 3 mL chloroform, vortex 1 min, centrifuge at 6000 rpm for 5 min. Remove organic layer and evaporate it to dryness at 45° under a stream of nitrogen. Vortex residue with 200 μL mobile phase for 10 s, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 30-40 μm C18 pellicular

Column: 150 × 3.9 Novapak C18
Mobile phase: MeCN:water 50:50 adjusted to pH 3.5 with glacial acetic acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 278

CHROMATOGRAM
Retention time: 3.8
Internal standard: mefenamic acid (6.3)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; dog

REFERENCE
Mohamed,F.A.; Jun,H.W.; Elfaham,T.H.; Sayed,H.A.; Hafez,E. An improved HPLC procedure for the quantitation of diclofenac in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 1065–1088.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize plasma with HCl and extract with dichloromethane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.6 ODS Hypersil
Mobile phase: MeCN:isopropanol:pH 7 sodium acetate buffer:water 21:6:20:53
Flow rate: 0.4
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: ranitidine

KEY WORDS
plasma; bioequivalence

REFERENCE
Van Gelderen,M.E.M.; Olling,M.; Barends,D.M.; Meulenbelt,J.; Salomons,P.; Rauws,A.G. The bioavailability of diclofenac from enteric coated products in healthy volunteers with normal and artificially decreased gastric acidity, *Biopharm.Drug Dispos.*, **1994**, *15*, 775–788.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 10 μg/mL flufenamic acid in MeCN + 4 mL MeCN, vortex for 1 min, centrifuge at 2500 rpm for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL mobile phase, vortex for 30 s, centrifuge at 11500 rpm for 2 min, inject a 140 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeCN:water 50:50 adjusted to pH 3.3 with glacial acetic acid

Flow rate: 2 for 13, increase to 2.7 over 4 min, maintain at 2.7 for 11 min, return to initial conditions
Injection volume: 140
Detector: UV 280

CHROMATOGRAM
Retention time: 6
Internal standard: flufenamic acid (10)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: nitrofenac (UV 275), metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Benoni,G.; Terzi,M.; Adami,A.; Grigolini,L.; Del Soldato,P.; Cuzzolin,L. Plasma concentrations and pharmacokinetic parameters of nitrofenac using a simple and sensitive HPLC method, *J.Pharm.Sci.*, **1995**, *84*, 93–95.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 600 μL 1 M phosphoric acid, vortex for 10 s, add 5 mL 30 ng/mL diphenylamine in hexane:isopropanol 95:5, vortex for 1 min, centrifuge at 1000 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 150 μL mobile phase, vortex for 30 s, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 10 μm Spherisorb ODS
Mobile phase: MeOH:buffer 68:32 (Buffer was 6.8 g/L sodium acetate adjusted to pH 4.2 with HCl.)
Flow rate: 1.4
Injection volume: 25
Detector: UV 274

CHROMATOGRAM
Retention time: 4.8
Internal standard: diphenylamine (6.4)
Limit of detection: 30 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: aspirin, chlorphenpyridamine, ciprofloxacin, ibuprofen, lomefloxacin, norfloxacin, ofloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Li,K.; Zhao,F.-L.; Yuan,Y.-S.; Tan,L. Determination of diclofenac sodium in human plasma by reversed-phase liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2205–2216.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 1 μg naproxen + 100 μL 5% zinc sulfate in water, vortex for 2 min, add 3 mL MeOH, vortex for 2 min, add 440 μL buffer, vortex for 1 min, centrifuge at 27° at 2000 g for 10 min, inject a 100 μL aliquot of the supernatant. (Buffer was 100 mM NaH_2PO_4 containing 10 mM sodium lauryl sulfate, adjust pH to 2.8 with orthophosphoric acid, filter (0.45 μm).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:water 35:65 containing 1 mM sodium lauryl sulfate and 10 mM NaH_2PO_4, pH adjusted to 2.8 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 10.6
Internal standard: naproxen (5.8)
Limit of detection: 30 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Mason,J.L.; Hobbs,G.J. A rapid high performance liquid chromatographic assay for the measurement of diclofenac in human plasma, *J.Liq.Chromatogr.*, **1995**, *18*, 2045–2058.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 275

CHROMATOGRAM

Retention time: 9.51
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebuto-

lol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 1 μg naproxen + 500 μL 500 mM HCl, vortex for 1 min, add 5 mL ethyl acetate, extract for 20 min, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeCN, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 5 μm C18 (Machery & Nagel)
Mobile phase: MeCN:water:acetic acid 50:50:0.1
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Internal standard: naproxen
Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Ramakrishna,S.; Fadnavis,N.W.; Diwan,P.V. Comparative pharmacokinetic evaluation of compressed suppositories of diclofenac sodium in humans, *Arzneimittelforschung*, **1996**, *46*, 175–177.

SAMPLE
Matrix: blood, CSF
Sample preparation: Clean Baker SPE-Octadecyl (C18) extraction column with 2 mL MeOH and 1 mL 1 M phosphoric acid. 0.5 mL Plasma or CSF + 2.5 ng (CSF) or 50 ng (plasma) pirprofen, shake, add 1 mL of 1 M phosphoric acid, add to column, wash twice with 1 mL 1 M phosphoric acid, wash twice with 1 mL water, elute with two 250 μL aliquots of MeOH. Evaporate MeOH at room temperature under a stream of nitrogen, dissolve residue in 100 μL mobile phase, inject 10-20 μL.

HPLC VARIABLES
Column: 80 × 4.6 3μm Perkin-Elmer C8
Mobile phase: MeCN:buffer 35:65 (Buffer was 50 mM sodium acetate adjusted to pH 3.00 with phosphoric acid.)
Flow rate: 1.2
Injection volume: 10-20
Detector: E, BAS LC-4B/17AT glassy carbon electrode, Ag/AgCl reference electrode, +0.95 V

CHROMATOGRAM
Retention time: 23
Internal standard: pirprofen (12)
Limit of detection: 0.7 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Zecca,L.; Ferrario,P.; Costi,P. Determination of diclofenac and its metabolites in plasma and cerebrospinal fluid by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *567*, 425–432.

SAMPLE
Matrix: blood, exudate
Sample preparation: 100 μL Plasma or exudate + 50 μL 50 μg/mL IS + 250 μL 900 mM phosphoric acid, vortex, add 2 mL hexane:isopropanol 90:10, rotate for 10 min, centrifuge, freeze. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 120 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 Hypersil ODS
Mobile phase: MeOH:MeCN:1% acetic acid 55:16:29
Flow rate: 1
Injection volume: 100
Detector: UV 282

CHROMATOGRAM
Internal standard: 2-(p-cyclohexen-1'-ylphenyl)propionic acid
Limit of detection: 100 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Stevens,A.J.; Martin,S.W.; Brennan,B.S.; McLachlan,A.; Gifford,L.A.; Rowland,M.; Houston,J.B. Regional drug delivery II: Relationship between drug targeting index and pharmacokinetic parameters for three non-steroidal anti-inflammatory drugs using the rat air pouch model of inflammation, *Pharm.Res.*, **1995**, *12*, 1987–1996.

SAMPLE
Matrix: blood, synovial fluid
Sample preparation: 0.5 mL Plasma or synovial fluid + 50 μL 24 μg/mL flurbiprofen + 200 μL 2 M HCl + 5 mL hexane, tumble 10 min on a rotary mixer, centrifuge at 10 000 g for 5 min. Remove organic layer and evaporate it to dryness under vacuum centrifugation. Reconstitute residue in 150 μL MeOH + 100 μL water, vortex mix, inject aliquot.

HPLC VARIABLES
Guard column: 20 × 2 Perisorb RP18 30-40 μm pellicular
Column: 125 × 4.6 5 μm Spherisorb ODS 1
Mobile phase: MeOH:water 63:37 adjusted to pH 3.3 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.5
Internal standard: flurbiprofen (9)
Limit of detection: <100 ng/mL

KEY WORDS
plasma

REFERENCE
Blagbrough,I.S.; Daykin,M.M.; Doherty,M.; Pattrick,M.; Shaw,P.N. High-performance liquid chromatographic determination of naproxen, ibuprofen and diclofenac in plasma and synovial fluid in man, *J.Chromatogr.*, **1992**, *578*, 251–257.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 50 μL 20 μM mefenamic acid + 200 μL 2 M HCl + 5 mL dichloromethane, rotate for 10 min, centrifuge at 5000 rpm for 8 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL plasma mobile phase, inject a 100 μL aliquot. Urine. 100 μL Urine + 100 μL 400 μg/mL ascorbic acid + 100 μL 5 M NaOH, vortex for 30 s, heat at 75° for 1 h, add 50 μL 20 μM mefenamic acid, add 500 μL 2 M HCl, add 5 mL dichloromethane, rotate for 10 min, centrifuge at 5000 rpm for 8 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL urine mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 4 μm Lichrocart C18 (Merck)
Column: 50 × 4 4 μm Lichrocart C18 (Merck)
Mobile phase: MeCN:100 mM pH 7.4 phosphate buffer:triethylamine 25:75:0.02 (plasma) or 20:80:0 (urine)
Flow rate: 1
Injection volume: 100
Detector: UV 282

CHROMATOGRAM
Internal standard: mefenamic acid

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: fluvastatin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Transon,C.; Leemann,T.; Vogt,N.; Dayer,P. In vivo inhibition profile of cytochrome P450TB (CYP2C9) by (±)-fluvastatin, *Clin.Pharmacol.Ther.*, **1995**, *58*, 412–417.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 22.115

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Powder tablets, shake (ca. 100 mg diclofenac) with 25 mL MeCN:water 25:75 for 30 min, centrifuge at 3500 rpm for 10 min.

HPLC VARIABLES
Column: 250 × 4.6 Chromatography Sciences Co. octadecylsilane bonded phase
Mobile phase: MeCN:THF:buffer 300:75:625 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 5.0 with 50 mM ammonium hydroxide.)
Flow rate: 1.5
Injection volume: 10

Detector: UV 229

CHROMATOGRAM
Retention time: 17
Limit of quantitation: 400 ng/mL

KEY WORDS
tablets

REFERENCE
Beaulieu,N.; Lovering,E.G.; Lefran[cois,J.; Ong,H. Determination of diclofenac sodium and related compounds in raw materials and formulations, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 698–701.

SAMPLE
Matrix: perfusate
Sample preparation: 250 μL mL Perfusate + 500 μL MeCN, vortex for 1 min, centrifuge for 10 min, inject a 150 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 mm long 5 μm Radpak C18
Mobile phase: MeOH:water:triethylamine 70:30:0.2 adjusted to pH 7 with concentrated phosphoric acid
Injection volume: 150
Detector: UV 273

CHROMATOGRAM
Limit of quantitation: 100 ng/mL

REFERENCE
Bassily,M.; Ghabrial,H.; Smallwood,R.A.; Morgan,D.J. Determinants of placental drug transfer: Studies in the isolated perfused human placenta, *J.Pharm.Sci.*, **1995**, *84*, 1054–1060.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 100-5C18
Mobile phase: MeOH:18.7 mM phosphoric acid 80:20
Flow rate: 1
Detector: UV 282

REFERENCE
Takahashi,K.; Suzuki,T.; Sakano,H.; Mizuno,N. Effect of vehicles on diclofenac permeation across excised rat skin, *Biol.Pharm.Bull.*, **1995**, *18*, 571–575.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 3 Ecocart LiChrospher 100 RP-18

Mobile phase: Isopropanol:100 mM KH_2PO_4:formic acid 54:100:0.1

Flow rate: 0.6
Detector: UV 254

CHROMATOGRAM
Retention time: 14.7
Limit of quantitation: 200-500 ng/mL

KEY WORDS
solutions acemetacin; flurbiprofen; indomethacin; lonazolac; ketoprofen; naproxen; piroxicam; sulindac; tenoxicam

REFERENCE
Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Zhang,Z.D. Preliminary results on the LC-separation of non-steroidal anti-inflammatory agents in conventional and narrow-bore RP set-ups applying columns with different internal diameters, *Biomed.Chromatogr.*, **1995**, *9*, 261–262.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:water 45:55, pH adjusted to 3.5 with acetic acid
Detector: UV 280

CHROMATOGRAM
Internal standard: phenylbutazone

OTHER SUBSTANCES
Also analyzed: indomethacin, clomethacin

REFERENCE
Guterres,S.S.; Fessi,H.; Barratt,G.; Puisieux,F.; Devissaguet,J.-P. Poly(D,L-lactide) nanocapsules containing non-steroidal anti-inflammatory drugs: Gastrointestinal tolerance following intravenous and oral administration, *Pharm.Res.*, **1995**, *12*, 1545–1547.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.68 (A), 9.97 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 250 μL Urine + 50 μL 12.5 μg/mL 4'-hydroxy-5-chlorodiclofenac in MeOH + 150 μL 5 M NaOH, vortex at medium speed for 5-10 s, heat at 70° for 1 h, cool to room temperature, neutralize with 1 M HCl, add 750 μL buffer, vortex, add 7 mL dichloromethane:isopropanol 95:5, shake horizontally at 180 cycles/min for 10 min, centrifuge at 750 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a vacuum at 30°, reconstitute the residue in 500 μL mobile phase containing 0.1% ascorbic acid, inject a 20 μL aliquot. (Buffer was 877 mL 1 M KH_2PO_4 and 123 mL 1 M Na_2HPO_4, pH 6.0.)

HPLC VARIABLES

Column: 150 mm long 5 μm ODS (Supelco)

Mobile phase: Gradient. MeOH:MeCN:buffer 57.5:0.3:42.2 for 12 min, to 57.5:1.5:41.0 over 2 min, maintain at 57.5:1.5:41.0 for 12 min, re-equilibrate at initial conditions for 4 min. (Buffer was 1.156 g $(NH_4)H_2PO_4$ in 1 L water adjusted to pH 2.66 with concentrated phosphoric acid.)

Flow rate: 1 for 12 min, to 1.6 over 2 min, 1.6 for 12 min, then 1

Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 21.7
Internal standard: 4'-hydroxy-5-chlorodiclofenac (18.66)
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

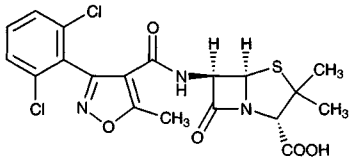
KEY WORDS

pharmacokinetics

REFERENCE

Sawchuk,R.J.; Maloney,J.A.; Cartier,L.L.; Rackley,R.J.; Chan,K.K.H.; Lau,H.S.L. Analysis of diclofenac and four of its metabolites in human urine by HPLC, *Pharm.Res.*, **1995**, *12*, 756–762.

Dicloxacillin



Molecular formula: $C_{19}H_{17}Cl_2N_3O_5S$
Molecular weight: 470.33
CAS Registry No.: 3116-76-5, 13412-64-1 (sodium salt monohydrate), 343-55-5 (sodium salt)
Merck Index: 3134
Lednicer No.: 1 413

SAMPLE

Matrix: blood
Sample preparation: 400 µL Serum + 400 µL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 µL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 11.7
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: cloxacillin, methicillin, nafcillin, oxacillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefamandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum

REFERENCE

Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MeCN and 1 mL 10 mM pH 2 Na_2HPO_4. 250 µL Plasma + 100 µL water, add 400 µL MeCN at -15° while vortexing, add 700 µL 10 mM pH 2 Na_2HPO_4, centrifuge at 8000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, elute with two 500 µL portions of MeCN:water 35:65 containing 10 mM Na_2HPO_4 (pH adjusted to 6 with phosphoric acid), inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

Column: 100 × 2 5 µm ODS Hypersil
Mobile phase: MeCN:water 40:60 containing 10 mM Na_2HPO_4, pH adjusted to 2 with orthophosphoric acid

Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 3.5
Internal standard: dicloxacillin

OTHER SUBSTANCES
Simultaneous: cloxacillin, floxacillin (flucloxacillin)

KEY WORDS
plasma; dicloxacillin is IS; SPE

REFERENCE
Hung,C.T.; Lim,J.K.C.; Zoest,A.R.; Lam,F.C. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design, *J.Chromatogr.*, **1988**, *425*, 331–341.

SAMPLE
Matrix: blood
Sample preparation: Condition a 55 × 5 100-200 mesh AG 50W-X8 (H^+) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 µL Serum + 600 µL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 µL effluent, collect the rest of the effluent. Remove a 450 µL aliquot and add it to 50 µL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 µL aliquot and add it to 15 µL 200 mM pH 6.0 phosphate buffer, add 35 µL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 µL 1 M HCl, inject a 5-10 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM pH 3.0 phosphate buffer 55:45
Flow rate: 1
Injection volume: 5-10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 9
Limit of detection: 45 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
derivatization; serum; SPE

REFERENCE
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 100 µL dicloxacillin in water + 25 µL glacial acetic acid + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the

supernatant and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 RP18 VeloSep (Brownlee)
Mobile phase: MeCN:10 mM pH 7 phosphate buffer 18:82
Flow rate: 1.2
Injection volume: 10-20
Detector: UV 220

CHROMATOGRAM
Retention time: 4.4
Internal standard: dicloxacillin
Limit of detection: 50 ng/mL
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Extracted: floxacillin
Simultaneous: phenobarbital
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, chloramphenicol, cyclosporine, digoxin, ethosuximide, gentamicin, lidocaine, nortriptyline, methotrexate, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin, metabolites
Interfering: carbamazepine, phenytoin

KEY WORDS
plasma; dicloxacillin is IS

REFERENCE
Charles,B.G.; Foo,C.C.; Gath,J. Rapid column liquid chromatographic analysis of flucloxacillin in plasma on a microparticulate pre-column, *J.Chromatogr.B*, **1994**, *660*, 186–190.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Plasma: Mix 50 μL MeCN and 50 μL plasma for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Urine: Mix 100 μL MeCN with 100 μL urine for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Tissue. Weigh out finely chopped tissue, suspend in 200 μL water. Mix with 200 μL MeCN, centrifuge at 10000 g for 15 min. Inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard C18 (Alltech)
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:50 mM pH 5.0 sodium dihydrogen phosphate 30:70
Flow rate: 1.0
Detector: UV 214

CHROMATOGRAM
Retention time: 13.9
Internal standard: dicloxacillin

OTHER SUBSTANCES
Extracted: penicillin G, flucloxacillin

KEY WORDS
dicloxacillin is IS; plasma; muscle; rat

REFERENCE
Cross,S.E.; Thompson,M.J.; Roberts,M.S. Distribution of systemically administered ampicillin, benzylpenicillin, and flucloxacillin in excisional wounds in diabetic and normal rats and effects of local topical vasodilator treatment, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1703–1710.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma, serum. 100 μL Plasma or serum + flucloxacillin + 100 μL 500 mM pH 2.2 citric acid buffer + 20 μL 500 mM HCl + 2.5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in mobile phase, inject an aliquot. Urine. Dilute urine with water, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 ODS pellicular
Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:20 mM pH 6.6 sodium acetate 34:100
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: k' 6.23
Internal standard: flucloxacillin (k' 4.19)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, oxacillin, cloxacillin

KEY WORDS
plasma; serum

REFERENCE
Thijssen,H.H.W. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 339–345.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out amount of bulk drug or capsule contents equivalent to 50 mg dicloxacillin, add 500 μL 30 mg/mL dimethyl phthalate in MeCN:water 50:50, make up to 50 mL with water, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:4% acetic acid 60:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: dimethyl phthalate (3.7)

OTHER SUBSTANCES
Noninterfering: excipients

KEY WORDS
capsules

REFERENCE
Hsu,M.-C.; Fann,Y.-J. Determination of cloxacillin preparations by liquid chromatography, *J.AOAC Int.*, **1992**, *75*, 26–29.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a solution of capsule contents or bulk drug in the mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeCN:1% acetic acid 39:61
Flow rate: 2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: degradation products, ampicillin

KEY WORDS
capsules; stability-indicating

REFERENCE
Al-Rashood,K. Simultaneous determination of ampicillin and dicloxacillin in pharmaceutical formulations by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2457–2465.

SAMPLE
Matrix: formulations
Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 mm long Co:Pell ODS
Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 26.8
Limit of detection: 3640 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin, cloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V

KEY WORDS
tablets; capsules; oral suspensions; injections

REFERENCE
Briguglio,G.T.; Lau-Cam,C.A. Separation and identification of nine penicillins by reverse phase liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 228–231.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out contents of amoxicillin/dicloxacillin capsules equivalent to 100 mg amoxicillin, add 10 mL water, stir magnetically for 10 min, filter, discard first 5 mL of the filtrate. 5 mL filtrate + 10 mL 1 mg/mL albuterol sulfate in water, make up to 100 mL with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeOH:20 mM ammonium acetate 50:50, pH adjusted to 5 with acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 6.066
Internal standard: albuterol sulfate (3.388)

OTHER SUBSTANCES
Simultaneous: amoxicillin

KEY WORDS
capsules

REFERENCE
el Walily,A.F.M.; el-Anwar,F.; Eid,M.A.; Awaad,H. High-performance liquid chromatographic and derivative ultraviolet spectrophotometric determination of amoxycillin and dicloxacillin mixtures in capsules, *Analyst*, **1992**, *117*, 981–984.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 30.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 2 mM phosphoric acid containing 8 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk

Sample preparation: Condition a 3 mL 500 mg Baker-10 C18 SPE cartridge (J.T. Baker) with 3 mL MeOH and 3 mL distilled water. Add 20 mL MeCN to 10 mL milk, vortex for 1 min, centrifuge at 1500 g for 10 min, concentrate the supernatant to 2-3 mL on a rotary evaporator at 40°, add to the SPE cartridge, dry the cartridge under reduced pressure for 3 min, elute with 1 mL MeOH, filter (0.45 μm) the eluate, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Kaseisorb LC ODS-300-5 (Tokyo Kasei)
Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 buffer 20:10:80 containing 5 mM sodium 1-decanesulfonate, adjusted to pH 3.5 with concentrated phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM

Retention time: 30
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: ampicillin, cloxacillin, nafcillin, penicillin G

KEY WORDS

SPE

REFERENCE

Takeba,K.; Fujinuma,K.; Miyazaki,T.; Nakazawa,H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 205–211.

SAMPLE

Matrix: milk
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 10 mL MeOH, 10 mL water, 5 mL 2% NaCl, and 5 mL 100 mM pH 8 phosphate extraction buffer. Add 30 mL 100 mM pH 8 phosphate extraction buffer to 5 mL milk, add 1.65 mL 1 M sulfuric acid to reach pH 4.0-4.5, vortex for 30 s, centrifuge at 2400 g for 10 min, add 600 μL 5 M NaOH to the supernatant to reach pH 8, vortex, centrifuge at 2400 g for 5 min. Add the supernatant to a reservoir attached to the SPE cartridge, pull through the SPE cartridge at 3 mL/min, remove the reservoir and elute with 1 mL MeCN:water 40:60. Add 500 μL derivatizing reagent to the eluate, vortex, heat at 65° for 10 min, cool to room temperature (protect from light), inject a 100 μL aliquot of the derivatized sample. (Prepare the 100 mM pH 8 phosphate extraction buffer as follows. Dissolve 15.6 g K_2HPO_4 dihydrate in 800 mL water, adjust pH to 8 with 10 M NaOH, make up to 1 L. Prepare the derivatizing reagent as follows. Weigh out 13.78 g 1,2,4-triazole, add 60 mL water, stir, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, dilute to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:MeOH:buffer 37:5:58 (Prepare the 100 mM pH 6.5 phosphate buffer containing 15 mM thiosulfate and 30 mM tetrabutylammonium hydrogen sulfate as follows. Weigh 4.969 g anhydrous NaH_2PO_4, 10.139 g Na_2HPO_4 dihydrate, 3.894 g sodium thiosulfate pentahydrate, and 10.186 g tetrabutylammonium hydrogen sulfate, dissolve in 800 mL water, adjust pH to 6.5 with 5 M NaOH, dilute to 1 L with water, mix thoroughly, filter under vacuum (0.45 μm).)
Flow rate: 1
Injection volume: 100
Detector: UV 340

CHROMATOGRAM
Retention time: 17
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: oxacillin, cloxacillin

KEY WORDS
derivatization; SPE

REFERENCE
Verdon,E.; Couedor,P. Determination of isoxazolylpenicillins residues in milk by ion-pair reversed-phase high-performance liquid chromatography after precolumn derivatization, *J.Chromatogr.B*, **1998**, *705*, 71–78.

SAMPLE
Matrix: milk
Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 10.73
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Simultaneous: penicillin G, methicillin, oxacillin, cloxacillin, penicillin V, nafcillin, phenethicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES
Column: 100 × 8 Radial-Pak 10 μm μBondapak C18
Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 21
Limit of detection: 0.3 ppm

OTHER SUBSTANCES
Simultaneous: penicillin V, ampicillin, methicillin, penicillin G, oxacillin, nafcillin, cloxacillin.

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 74.90

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM
Retention time: 40

Internal standard: penicillin V (28.5)
Limit of detection: 2.7 ng/mL
Limit of quantitation: 3.7 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin, ampicillin, cloxacillin, oxacillin, penicillin G

KEY WORDS
derivatization; cow; SPE

REFERENCE
Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: methicillin, penicillin G, penicillin V, cloxacillin, nafcillin, carbenicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)

Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 28.8

OTHER SUBSTANCES
Simultaneous: carbenicillin, cephapirin, cloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 25:65
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to stand for 30 min. Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 μL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on

to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES

Column: A 4 × 4 5 μm LiChrospher 100 RP-18e; B 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 13.0
Limit of detection: 4.6 ng

OTHER SUBSTANCES

Extracted: cloxacillin, oxacillin, penicillin V, penicillin G

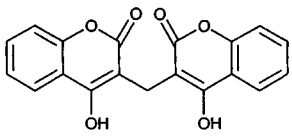
KEY WORDS

post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE

Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

Dicumarol



Molecular formula: $C_{19}H_{12}O_6$
Molecular weight: 336.30
CAS Registry No.: 66-76-2
Merck Index: 3140
Lednicer No.: 1 147

SAMPLE

Matrix: formulations
Sample preparation: Powder tablets, make a 250 μg/mL solution in 10 mM NaOH. Remove a 5 mL aliquot and make it up to 25 mL with mobile phase, filter, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: THF:MeOH:water:acetic acid 35:10:65:0.1
Flow rate: 1.5
Injection volume: 20
Detector: UV 311

OTHER SUBSTANCES

Also analyzed: warfarin, phenprocoumon

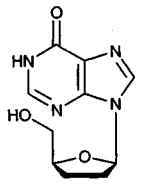
KEY WORDS

tablets

REFERENCE

Moore,E.S. Liquid chromatographic determination of coumarin anticoagulants in tablets: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 834–836.

Didanosine



Molecular formula: $C_{10}H_{12}N_4O_3$
Molecular weight: 236.23
CAS Registry No.: 69655-05-6
Merck Index: 3148
Lednicer No.: 5 146

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. 50 μL Plasma or amniotic fluid + 50 μL 5 μg/mL 3-hydroxyacetamidophenol in water, add to SPE cartridge, wash twice with 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate under vacuum, reconstitute the residue in 100 μL MeCN:water 6:94, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: Gradient. A was MeCN:pH 4.0 ammonium phosphate buffer 6:94. B was MeCN:pH 4.0 ammonium phosphate buffer 25:75. A:B from 100:0 to 100:0 over 20 min, to 100:0 over 5 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: antipyrine

KEY WORDS

plasma; monkey; pharmacokinetics; SPE

REFERENCE

Pereira,C.M.; Nosbisch,C.; Winter,H.R.; Baughman,W.L.; Unadkat,J.D. Transplacental pharmacokinetics of dideoxyinosine in pigtailed macaques, *Antimicrob.Agents Chemother.*, **1994**, *38*, 781–786.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Supelclean C18 SPE cartridge with MeOH, water, and two aliquots of 10 mM pH 8.0 phosphate buffer. Add 500 μL plasma then 1 mL 5 μg/mL IS in Na_2HPO_4 buffer to the SPE cartridge. Wash with 10 mM pH 8.0 phosphate buffer and water. Dry cartridge under vacuum and elute with 2 aliquots of MeOH. Evaporate the eluate to dryness and reconstitute the residue in 10 mM pH 8.0 phosphate buffer. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelguard LC-18s
Column: 250 × 4.6 5 μm Supelcosil LC-18s
Mobile phase: MeOH:100 mM pH 5.0 phosphate buffer 20:80
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Internal standard: d4C

KEY WORDS
dog; plasma; pharmacokinetics; SPE

REFERENCE
Sinko,P.J.; Sutyak,J.P.; Leesman,G.D.; Hu,P.; Makhey,V.D.; Yu,H.; Smith,C.L. Oral absorption of anti-AIDS nucleoside analogues: 3. Regional absorption and in vivo permeability of 2',3'-dideoxyinosine in an intestinal-vascular access port (IVAP) dog model, *Biopharm.Drug Dispos.*, **1997**, *18*, 697–710.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bakerbond C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. Add 500 μL plasma to the SPE cartridge, wash with 1 mL water, allow to dry under vacuum for 10 min, elute with two 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex for 30 s, centrifuge at 800 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 LiChroCART 4-4 RP-8 (Merck)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeOH:5 mM pH 6.8 phosphate buffer 10:90
Flow rate: 1
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 10.5
Internal standard: didanosine

OTHER SUBSTANCES
Extracted: stavudine

KEY WORDS
plasma; rat; human; didanosine is IS; SPE

REFERENCE
Burger,D.M.; Rosing,H.; van Gijn,R.; Meenhorst,P.L.; van Tellingen,O.; Beijnen,J.H. Determination of stavudine, a new antiretroviral agent, in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1992**, *584*, 239–247.

SAMPLE
Matrix: blood
Sample preparation: Condition a Toyopak ODS M SPE cartridge (Tosoh, Tokyo) with 6 mL MeOH, 12 mL water, and 2 mL 100 mM pH 4.5 phosphate buffer. 100 μL Plasma + 10 μL 85 μM IS + 890 μL 100 mM pH 4.5 phosphate buffer, mix, add to the SPE cartridge at 120-150 μL/min, wash with 2 mL 100 mM ph 4.5 phosphate buffer, wash with 2 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL dry pyridine, add 900 μL 3 mM reagent in dry benzene (Caution! Benzene is a carcinogen!), heat at 100° in the dark for 50 min, cool, evaporate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, inject a 20 μL aliquot. (The reagent was 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (Dojindo Laboratories, Kumamoto, Japan). Synthesis is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Bueh-

ler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°) (Anal. Sci. 1989, 5, 525).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS-80TM
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 35:65
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 475

CHROMATOGRAM

Retention time: 14.6
Internal standard: 3'-deoxythymidine (21.5)
Limit of detection: 1.3 pmole

OTHER SUBSTANCES

Extracted: 2',3'-dideoxyadenosine

KEY WORDS

derivatization; rat; plasma; pharmacokinetics; SPE

REFERENCE

Nagaoka,H.; Nohta,H.; Saito,M.; Ohkura,Y. Determination of 2',3'-dideoxyinosine and 2',3'-dideoxyadenosine in rat plasma by high-performance liquid chromatography with precolumn fluorescence derivatization, *Chem.Pharm.Bull.(Tokyo)*, **1992**, *40*, 2202–2204.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 400 μL ethyl acetate:MeCN 50:50, vortex for 30 s. Remove a 200 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B

Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: MeOH:pH 7.0 phosphate buffer 5:95
Flow rate: 1
Injection volume: 10
Detector: UV 250

OTHER SUBSTANCES
Also analyzed: zalcitabine (UV 270)

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Mirchandani,H.L.; Chien,Y.W. Intestinal absorption of dideoxynucleosides: Characterization using a multiloop in situ technique, *J.Pharm.Sci.*, **1995**, *84*, 44–48.

SAMPLE
Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μL serum while centrifuging at 17000 g for 1.5 h, inject a 50 μL aliquot of the clear ultrafiltrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: Isopropanol:20 mM pH 5 sodium citrate 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 5.32
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: zalcitabine, zidovudine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Rosell-Rovira,M.L.; Pou-Clavé,L.; Lopez-Galera,R.; Pascual-Mostaza,C. Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 89–92.

SAMPLE
Matrix: blood, CSF
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 6 mL MeOH and 12 mL water. 1 mL Plasma or CSF + 2'-deoxyadenosine, add to the SPE cartridge, wash with 1 (CSF) or 2 (plasma) mL water, elute slowly with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, filter (Ultrafree-MC 0.45 μm) while centrifuging, inject an aliquot of the filtrate. (CSF can also be injected directly.)

HPLC VARIABLES
Column: 100 × 8 Nova-Pak radial compression
Mobile phase: MeOH:10 mM ammonium phosphate 10:90
Flow rate: 1.5

Detector: UV 248

CHROMATOGRAM
Retention time: 4.6
Internal standard: 2'-deoxyadenosine (5.3)
Limit of detection: 100 nM

OTHER SUBSTANCES
Extracted: 2',3'-dideoxyadenosine (UV 260)

KEY WORDS
plasma; monkey; SPE; pharmacokinetics

REFERENCE
Hawkins,M.E.; Mitsuya,H.; McCully,C.M.; Godwin,K.S.; Murakami,K.; Poplack,D.G.; Balis,F.M. Pharmacokinetics of dideoxypurine nucleoside analogs in plasma and cerebrospinal fluid of Rhesus monkeys, *Antimicrob.Agents Chemother.*, **1995**, *39*, 1259–1264.

SAMPLE
Matrix: blood, cell suspensions, perfusate
Sample preparation: Centrifuge cellular suspensions at 17000 g for 5 min, inject a 25 μL aliquot. Centrifuge perfusion fluid at 17000 g for 5 min, inject a 50 μL aliquot. Dilute 1 mL plasma with 1 mL saturated ammonium sulfate, vortex for 30 s, centrifuge at 3000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm Phenyl Hypersil NC-04
Mobile phase: MeOH:1.4 g/L sodium acetate 15:85, adjusted to pH 6.55
Flow rate: 1
Injection volume: 25-50
Detector: UV 250

CHROMATOGRAM
Retention time: 8
Limit of detection: 25 ng/mL

KEY WORDS
plasma

REFERENCE
Frijus-Plessen,N.; Michaelis,H.C.; Foth,H.; Kahl,G.F. Determination of 3'-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 3'-fluoro-3'-deoxythymidine and 2',3'-dideoxyinosine in biological samples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 101–107.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 100 mM pH 6.9 phosphate buffer. 100 μL Plasma + 400 μL 10 μg/mL IS in 100 mM pH 6.9 phosphate buffer, add to the SPE cartridge, let stand for 1 min, wash with two 1 mL portions of 100 mM pH 6.9 phosphate buffer, elute with 1 mL MeOH:5 mM pH 6.9 phosphate buffer 75:25, concentrate the eluate to about 150 μL under a stream of nitrogen, inject an aliquot. Urine. 100 μL Urine + 400 μL 10 μg/mL IS in 100 mM pH 6.9 phosphate buffer, mix, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:10 mM phosphate buffer adjusted to pH 6.9 with dilute phosphoric acid 4:96
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 280

CHROMATOGRAM
Retention time: 8
Internal standard: 5'-deoxy-5-fluorouridine (5)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; rat; pharmacokinetics; SPE

REFERENCE
Wientjes,M.G.; Au,J.L.-S. High-performance liquid chromatographic analysis of 2',3'-dideoxyinosine in biological samples, *J.Chromatogr.*, **1991**, *563*, 400–406.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a 3 mL Bond Elut SPE cartridge with one volume MeOH and one volume water. 25-400 μL Plasma + 50 μL 5 μg/mL 3-acetamidophenol in water, make up to 500 μL with water, add to SPE cartridge, wash with two 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 100 μL MeCN:water 6:94, inject a 50 μL aliquot. Urine. 1 mL Urine + 49 mL water + 1 mL 100 μg/mL 3-acetamidophenol, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 C18 (Alltech)
Column: 250 × 4.6 Ultrasphere C18
Mobile phase: MeCN:50 mM pH 4.0 ammonium phosphate 6:94
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2
Internal standard: 3-acetamidophenol (14.0)
Limit of detection: 500 ng/mL (plasma)

KEY WORDS
plasma; monkey; SPE; pharmacokinetics

REFERENCE
Ravasco,R.J.; Unadkat,J.D.; Tsai,C.C. A high-performance liquid chromatographic assay for dideoxyinosine in monkey plasma and urine, *J.Pharm.Sci.*, **1992**, *81*, 690–691.

SAMPLE
Matrix: cell suspensions
Sample preparation: 1 mL Cell suspension + 500 μL ice-cold MeCN + 500 μL water, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES

Column: Partisil-10 SAX
Mobile phase: Gradient. A was 30 mM pH 4.8 ammonium phosphate. B was MeCN:700 mM pH 4.6 ammonium phosphate 10:90. A:B 100:0 for 5 min then a convex gradient to 75:25 over 10 min then a convex gradient to 0:100 over 15 min, stay at 0:100 for 15 min
Flow rate: 1.7
Detector: UV 254

CHROMATOGRAM

Retention time: 9.0

OTHER SUBSTANCES

Extracted: metabolites, ATP

REFERENCE

Mukherji,E.; Au,J.L.-S.; Mathes,L.E. Differential antiviral activities and intracellular metabolism of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine in human cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1573–1579.

SAMPLE

Matrix: intestinal mucosal homogenate
Sample preparation: 400 μL Homogenate mixture + 400 μL 250 mM NaCN, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18S (Supelco)
Column: 250 × 4.6 Suplecosil LC-18S
Mobile phase: MeOH:100 mM potassium phosphate 20:80 or MeOH:33 mM potassium phosphate 6.7:93.3
Flow rate: 1
Detector: UV 254

KEY WORDS

rat

REFERENCE

Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

SAMPLE

Matrix: perfusate
Sample preparation: Dilute perfusate with an equal volume of 15 mM pH 8 HEPES buffer, centrifuge at 2000 g for 2 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18S (Supelco)
Column: 250 × 4.6 Supelcosil LC-18S
Mobile phase: MeOH:100 mM potassium phosphate 20:80
Flow rate: 1
Detector: UV 254

KEY WORDS

rat; rabbit; pharmacokinetics

REFERENCE

Sinko,P.J.; Hu,P.; Waclawski,A.P.; Patel,N.R. Oral absorption of anti-AIDS nucleoside analogues. 1. Intestinal transport of didanosine in rat and rabbit preparations, *J.Pharm.Sci.*, **1995**, *84*, 959–965.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 220 × 4.6 5μm Brownlee C18
Mobile phase: MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 15:85, pH adjusted to 4.0 with 85% phosphoric acid (A) or MeCN:MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 3:15:85, pH adjusted to 4.0 with 85% phosphoric acid (B)
Column temperature: -10
Flow rate: 0.7
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 22 (A), 15 (B)

OTHER SUBSTANCES
Simultaneous: stavudine

REFERENCE
Stancato,F.A.; Srinivas,N.R.; Knupp,C.A. Effect of temperature on the high-performance liquid chromatographic separation of the anti-HIV agents, didanosine and stavudine, *Biomed.Chromatogr.*, **1996**, *10*, 29–31.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 10:90
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.85

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type anti-HIV drugs. 1. Stability studies for hairless rat skin permeation, *J.Pharm.Sci.*, **1995**, *84*, 1061–1066.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC18 DB
Mobile phase: MeCN:10 mM ammonium acetate 4:96
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Simultaneous: impurities, adenosine, deoxyinosine, dideoxyadenosine, hypoxanthine, inosine

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

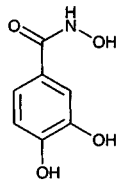
SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 10:90
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.85

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type Anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation, *J.Pharm.Sci.*, **1996**, *85*, 214–219.

Didox



Molecular formula: $C_7H_7NO_4$
Molecular weight: 169.13
CAS Registry No.: 69839-83-4

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 0.1 mM solution in 10 mM KH_2PO_4, adjust pH to 6 with a few drops 5 M KOH or phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Supelcosil LC18
Mobile phase: MeOH:buffer 5:95 (Buffer was 0.05% triethylamine adjusted to pH 6 with 50 mM phosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 255

OTHER SUBSTANCES

Simultaneous: amidox, trimidox

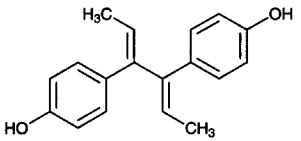
KEY WORDS

comparison with DC polarography and UV spectrophotometry

REFERENCE

Romanova,D.; Vachalkova,A.; Szekeres,T.; Elford,H.L.; Novotny,L. The new inhibitors of ribonucleotide reductase -comparison of some physico-chemical properties, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 951–956.

Dienestrol



Molecular formula: $C_{18}H_{18}O_2$
Molecular weight: 226.36
CAS Registry No.: 84-17-3, 13029-44-2 (E,E)
Merck Index: 3153
Lednicer No.: 1 102

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 4.70

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: diethylstilbestrol, trenbolone, nandrolone, zeranol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE

Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE

Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES

Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

CHROMATOGRAM

Retention time: 7 (α), 10 (β)
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: trans-diethylstilbestrol, meso-hexestrol

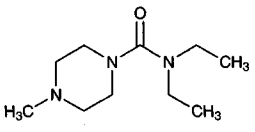
KEY WORDS

cow

REFERENCE

Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

Diethylcarbamazine



Molecular formula: $C_{10}H_{21}N_3O$
Molecular weight: 199.30
CAS Registry No.: 90-89-1, 1642-54-2 (citrate)
Merck Index: 3165
Lednicer No.: 1 278

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

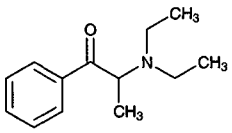
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protripty-

line, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Diethylpropion



Molecular formula: $C_{13}H_{19}NO$
Molecular weight: 205.30
CAS Registry No.: 90-84-6, 134-80-5 (HCl)
Merck Index: 3175

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 8.688

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.53

OTHER SUBSTANCES
Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: pemoline, benzphetamine, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin,

laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: phenylpropanolamine, ephedrine, amphetamine, methamphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

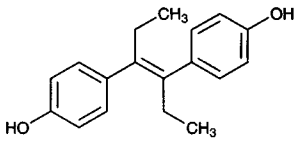
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Diethylstilbestrol



Molecular formula: $C_{18}H_{20}O_2$
Molecular weight: 268.36
CAS Registry No.: 56-53-1
Merck Index: 3177
Lednicer No.: 1 101

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 5 mL MTBE, vortex for 20 s, centrifuge at 170 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and put it into a clean tube (twice), evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL MeCN:water 45:55, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 3.2 5 μm Hypersil C8
Column: 150 × 3.2 5 μm Hypersil C8
Mobile phase: MeOH:30 mM pH 6 potassium phosphate buffer 58:42 (After 25 min wash column with MeOH:water 95:5 for 7 min, re-equilibrate for 13 min.)
Flow rate: 0.4
Injection volume: 50
Detector: UV 227

CHROMATOGRAM

Retention time: 17
Internal standard: diethylstilbestrol

OTHER SUBSTANCES

Extracted: paclitaxel

KEY WORDS

plasma; diethylstilbestrol is IS

REFERENCE

Sonnichsen,D.S.; Liu,Q.; Schuetz,E.G.; Schuetz,J.D.; Pappo,A.; Relling,M.V. Variability of human cytochrome P450 paclitaxel metabolism, *J.Pharmacol.Exp.Ther.*, **1995**, *275*, 566–575.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.882

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Pellets. Grind 3 from each lot and extract with 10 mL MeOH with vigorous shaking for 20 min. Take a 100 μL aliquot and dilute it with 1 mL 5 μg/mL dexamethasone in MeOH. Inject a 10 μL aliquot. Oils. Dilute 1 mL in 10 mL of diethyl ether and pass 1 mL through a 5 cm Sephadex LH-20 column. Elute the drug with 8 mL dichloromethane:MeCN 94:6. Discard the first 3 mL and evaporate the remaining 5 mL under nitrogen. Dissolve the residue in 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 1.0
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 5.4 (trans), 8.3 (cis)
Internal standard: dexamethasone (2.9)
Limit of detection: 200 ng/mL
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: hexestrol

KEY WORDS
comparison with TLC; pellets; oils

REFERENCE
Nascimento,E.S.; Salvadori,M.C.; Ribeiro-Neto,L.M. Determination of synthetic estrogens in illegal veterinary formulations by HPTLC and HPLC, *J.Chromatogr.Sci.*, **1996**, *34*, 330–333.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 4.68

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: trenbolone, nandrolone, zeranol, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine,

cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize 2.5 g tissue with 10 mL acetone for 20 s, sonicate for 5 min, centrifuge at 3200 rpm. Decant the supernatant into a silanized tube. Add 8 mL acetone to the pellet and repeat the extraction. Combine the supernatants. Add to a 5 mL pipette tip containing 1.5 g alumina (80-200 mesh, Brockman activity 1) followed by an Econo-Column filled with 1.0 g AGMP-1 resin (Bio-Rad), allow to pass through by gravity. Wash with four 1 mL portions of acetone:water 95:5. Remove the alumina column, wash the ion-exchange column with 1 mL acetone:water 95:5, elute with four 1 mL portions of 10% acetic acid in acetone. Evaporate the combined eluates to dryness with nitrogen at 40°. Add 500 μL water to the residue, extract twice with 2 mL potions of ether. Combine the ether layers and evaporate them to dryness. Reconstitute the residue in mobile phase B. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco silica

Mobile phase: Gradient. A was hexane. B was MeOH:hexane:2-propanol 45:40:15. A:B from 100:0 to 60:40 over 15 min.
Flow rate: 2.0
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 10.81
Limit of detection: 10 ng

OTHER SUBSTANCES
Extracted: estradiol, zeralenone, zeranol
Simultaneous: estrone, zeralenol, zeralanone

KEY WORDS
chicken; muscle; normal phase; SPE

REFERENCE
Medina,M.B.; Sherman,J.T. High performance liquid chromatographic separation of anabolic oestrogens and ultraviolet detection of 17β-oestradiol, zeranol, diethylstilboestrol or zearalenone in avian muscle tissue extracts, *Food Addit.Contam.*, **1986**, *3*, 263–272.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH:water 85:15, collect all eluates and pass onto Amberlite column, wash with 1 mL MeOH, 1 mL 1 M HCl, aspirate with vacuum for 1 min, elute with 2 mL 30 mM HCl in MeCN:MeOH 20:80. Evaporate eluate to dryness with nitrogen at 40°, take up in 100 μL mobile phase, inject 50 μL aliquot

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 48:52
Flow rate: 1.2
Injection volume: 50
Detector: E, Coulochem 5100A, detector 1 0.05 V, detector 2 0.30 V

CHROMATOGRAM
Retention time: 11
Limit of detection: 1 ng/g

KEY WORDS
muscle; liver; chicken; ox

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM
Retention time: 13
Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: dexamethasone, medroxyprogesterone, melengestrol acetate, trenbolone, triamcinolone acetonide, zeranol

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE
Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES
Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

CHROMATOGRAM
Retention time: 7.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: 17α-methyltestosterone, 17β-trenbolone, zeranol, medroxyprogesterone, nandrolone

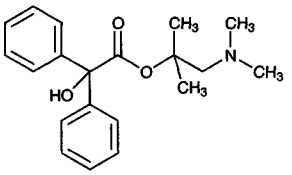
KEY WORDS

cow

REFERENCE

Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

Difemerine



Molecular formula: $C_{20}H_{25}NO_3$
Molecular weight: 327.42
CAS Registry No.: 80387-96-8, 70280-88-5 (HCl)
Merck Index: 3180

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.222

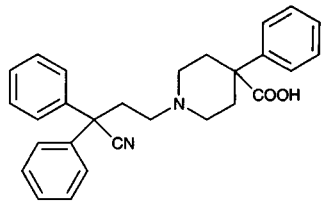
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Difenoxin



Molecular formula: $C_{28}H_{28}N_2O_2$
Molecular weight: 424.54
CAS Registry No.: 28782-42-5
Merck Index: 3183
Lednicer No.: 2 331

SAMPLE

Matrix: blood
Sample preparation: 900 μL Plasma + 100 μL mobile phase, add to a C18 Sep Pak SPE cartridge at 1 mL/min, wash with 4 mL MeCN:buffer 10:90 at 1 mL/min, elute with 4 mL MeCN:buffer 40:60 at 5 mL/min, inject a 100 μL aliquot of the eluate. (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 30 μm Ultracarb ODS (Phenomenex)
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)
Column temperature: 28
Flow rate: 1.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 32
Internal standard: difenoxin

OTHER SUBSTANCES

Extracted: methadone

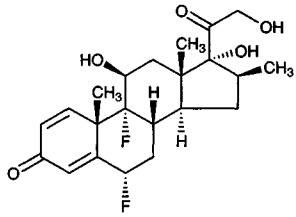
KEY WORDS

plasma; SPE; rat; difenoxin is IS

REFERENCE

Pierce,T.L.; Murray,A.G.W.; Hope,W. Determination of methadone and its metabolites by high performance liquid chromatography following solid-phase extraction in rat plasma, *J.Chromatogr.Sci.*, **1992**, *30*, 443–447.

Diflorasone



Molecular formula: $C_{22}H_{28}F_2O_5$
Molecular weight: 410.46
CAS Registry No.: 2557-49-5, 33564-31-7 (diacetate)
Merck Index: 3186

SAMPLE

Matrix: bulk, formulations
Sample preparation: Cream. Weigh out cream containing 1 mg diflorasone diacetate, add 30 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Ointment. Weigh out ointment containing 0.5 mg diflorasone diacetate, add 15 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Bulk. Dissolve 1.5 mg bulk drug in 50 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm silica gel (Perkin-Elmer part 0258-1500)
Mobile phase: Butyl chloride:dichloromethane:THF:acetic acid 70:25:2:3 (Butyl chloride and dichloromethane were saturated with water.)
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 11 (diflorasone diacetate)
Internal standard: isoflupredone acetate (24)

OTHER SUBSTANCES

Simultaneous: related compounds

KEY WORDS

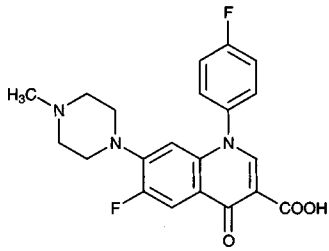
cream; ointment; normal phase

REFERENCE

Shaw,M.C.; Vanderwielen,A.J. Liquid chromatographic assay for diflorasone diacetate in cream and ointment formulations, *J.Pharm.Sci.*, **1984**, *73*, 1606–1608.

Difloxacin

Molecular formula: $C_{21}H_{19}F_2N_3O_3$
Molecular weight: 399.40
CAS Registry No.: 98106-17-3, 91296-86-5 (HCl)
Merck Index: 3187



SAMPLE

Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 8.51
Internal standard: rosoxacin (4.41)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: milk
Sample preparation: Condition a 500 mg Bond Elut LRC PRS SPE cartridge with 5 mL MeOH and 5 mL extracting solution 65:35. Add 25 mL extracting solution to 5 mL milk, shake for 15 s, add 4 g anhydrous sodium sulfate, shake for 15 s, centrifuge at 3000 rpm at 5° for 5 min. Remove the supernatant and repeat the extraction with 25 mL extracting solution as before except do not add any more sodium sulfate, mix mechanically, centrifuge, combine the supernatants, add 25 mL 1% acetic acid, shake for 10-15 s. Freeze for 30 min to facilitate precipitation, centrifuge at 2500 rpm at 5° for 10 min. Add 75 mL to the SPE cartridge, pass the entire sample through the cartridge, then add 2 mL MeOH, wash with 5 mL water, wash with 2 mL MeOH. Elute with 2.5 mL 25% ammonium hydroxide-MeOH. Evaporate to dryness under nitrogen at 55°, dissolve the residue in 2 mL 1% acetic acid, sonicate for 1 min, vortex for 20 s, filter (0.45 μm), inject an aliquot. (Extracting solution was 1% aqueous acetic acid:EtOH 1:99.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil
Mobile phase: MeCN:2% acetic acid 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 450, with a 418 nm cut-off filter

CHROMATOGRAM
Retention time: 6.1
Limit of detection: 0.7 ppb
Limit of quantitation: 5 ppb

OTHER SUBSTANCES
Extracted: ciprofloxacin, enrofloxacin, sarafloxacin

KEY WORDS
SPE

REFERENCE
Roybal,J.E.; Pfenning,A.P.; Turnipseed,S.B.; Walker,C.C.; Hurlbut,J.A. Determination of four fluoroquinolones in milk by liquid chromatography, *J.AOAC Int.*, **1997**, *80*, 982–987.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC PRS SPE cartridge with 2 mL MeOH and 2 mL equilibrating solution. 2 g Catfish muscle + 18 mL extracting solution, homogenize for 20 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add another 18 mL extracting solution to the pellet and homogenize again, centrifuge at 3000 rpm for 5 min, combine the supernatants. Add 20 mL 1% glacial acetic acid, freeze for 30 min, centrifuge at 2500 rpm at 4° for 10 min. Add the extracts to the SPE cartridge, wash with 2 mL MeOH, 5 mL water, and 2 mL MeOH. Let the SPE cartridge dry for 30 s. Elute with 2 mL MeOH:30% ammonium hydroxide 80:20, dry the eluate under nitrogen at 50°. Reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject an aliquot. (The extracting solution was EtOH:water:glacial acetic acid 98:1:1. The equilibrating solution was extracting solution:1% glacial acetic acid 35:20.)

HPLC VARIABLES
Column: 150 × 2 5 μm Inertsil Phenyl
Mobile phase: MeCN:2% formic acid 14:86
Column temperature: 40
Flow rate: 0.35
Injection volume: 50
Detector: MS, Hewlett-Packard 5989, Model 59987A electrospray, nitrogen drying gas 40 mL/min, 260°, nebulizing gas nitrogen, 80 psi, m/z 299

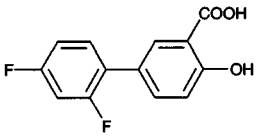
CHROMATOGRAM
Retention time: 8.52-8.70
Limit of detection: 10 ppb
Limit of quantitation: 20 ppb

OTHER SUBSTANCES
Extracted: sarafloxacin

KEY WORDS
catfish; muscle; SPE

REFERENCE
Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Pfenning,A.P.; Hurlbut,J.A. Confirmation of fluoroquinolones in catfish muscle by electrospray liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1998**, *81*, 554–562.

Diflunisal



Molecular formula: $C_{13}H_8F_2O_3$
Molecular weight: 250.20
CAS Registry No.: 22494-42-4
Merck Index: 3190
Lednicer No.: 2 85

SAMPLE

Matrix: bile, tissue
Sample preparation: Acidify bile or crushed liver to pH 2.0 with 1 M HCl and extract with 2.5 volumes of ether. Cool the mixture to -20°. Remove the ether fraction and evaporate it to a final volume of 100 µL. Add 20 µL 35% perchloric acid and 200 µL 0.004% IS in MeCN. Vortex and centrifuge at 9000 g for 5 min. Inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5µm Brownlee C18 (Applied Biosystems)
Mobile phase: MeCN:0.03% phosphoric acid:triethylamine 64:35:1, pH 2.0
Flow rate: 1
Injection volume: 20
Detector: UV 237

CHROMATOGRAM

Internal standard: salicylic acid

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pharmacokinetics; liver

REFERENCE

Hung,D.Y.; Mellick,G.D.; Anissimov,Y.G.; Weiss,M.; Roberts,M.S. Hepatic disposition and metabolite kinetics of a homologous series of diflunisal esters, *J.Pharm.Sci.*, **1998**, *87*, 943–951.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 200 µL 250 µg/mL naproxen in MeOH, mix on a whirlmixer, add 5 mL diethyl ether:n-hexane 1:1 + 700 µL 1.5 M HCl, shake 30 min, centrifuge at 1500 g for 15 min. Remove organic layer and evaporate it to dryness at 30° under a stream of dry air, take up residue in 1 mL MeOH, inject 10 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5µm LiChrosorb RP-8
Mobile phase: MeOH:water 50:50 containing 10 mM tetramethylammonium hydrogen sulfate and 10 mM tris(hydroxymethyl)aminomethane (Tris)
Column temperature: 32
Flow rate: 1.4
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 4.11
Internal standard: naproxen (2.55)
Limit of detection: < 5000 ng/mL

KEY WORDS
plasma

REFERENCE
Van Loenhout,J.W.; Ketelaars,H.C.; Gribnau,F.W.; Van Ginneken,C.A.; Tan,Y. Rapid high-performance liquid chromatographic method for the quantitative determination of diflunisal in plasma, *J.Chromatogr.*, **1980**, *182*, 487–491.

SAMPLE
Matrix: blood
Sample preparation: Wash a Sep-Pak C18 cartridge with 2 mL MeOH, 5 mL water, and 1 mL 0.25 mM pH 3.0 ammonium phosphate buffer. 20-200 μL Plasma + 100 μL MeOH + 20 μL 50 μg/mL indomethacin in MeOH + 100 μL 0.25 mM pH 3.0 ammonium phosphate buffer + 100 μL water, vortex for 2 min, centrifuge at 1800 g for 10 min. Add the supernatant to the cartridge, wash with 5 mL water, elute twice with 5 mL portions of MeOH. Evaporate eluate to dryness under vacuum, dissolve the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP18
Mobile phase: MeOH:buffer 75:25 (Buffer prepared by diluting 0.25 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)
Injection volume: 20
Detector: E ESA Coulochem Model 5100 A, + 0.9 V

CHROMATOGRAM
Retention time: 8
Internal standard: indomethacin (14.6)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Also analyzed: naproxen, piroxicam, sulindac

KEY WORDS
plasma

REFERENCE
Kazemifard,A.G.; Moore,D.E. Liquid chromatography with amperometric detection for the determination of non-steroidal anti-inflammatory drugs in plasma, *J.Chromatogr.*, **1990**, *533*, 125–132.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL MeCN:water 80:20 + 50 μL 100 μg/mL IS in MeCN:water 80:20 + 250 μL 0.1 M phosphoric acid + 750 μL MeCN, vortex 10 s, centrifuge at 2000 g for 15 min, inject aliquot of supernatant. Urine. 50 μL Urine + 50 μL MeCN:water 80:20 + 100 μL 150 μg/mL IS in MeCN:water 80:20 + 150 μL 70% perchloric acid, heat at 90° for 1 h, cool to room temperature, add 500 μL 5 M NaOH + 200 μL MeCN, vortex, centrifuge at 2000 g for 15 min, inject aliquot of supernatant.

HPLC VARIABLES
Column: 50 × 4.6 3 μm Sepralyte C18
Mobile phase: MeOH:buffer 58:42 (Buffer was 50 mM ammonium dihydrogen phosphate + 10 mM phosphoric acid, pH 3.5.)
Column temperature: 65
Flow rate: 1.5
Injection volume: 10 (plasma) 12 (urine)
Detector: F ex 269 em 418 (slits 15 nm)

CHROMATOGRAM
Retention time: 1.4
Internal standard: 5-(2'-chloro-4'-fluorophenyl)-2-hydroxybenzoic acid (1.9)
Limit of detection: 2000 ng/mL

KEY WORDS
plasma

REFERENCE
Schwartz,M.; Chiou,R.; Stubbs,R.J.; Bayne,W.F. Determination of diflunisal in human plasma and urine by fast high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *380*, 420–424.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Acidify 5 mL serum to pH 4 with 25 μL of 50% citric acid, dilute with an equal volume MeCN, centrifuge at 18 000 g for 4 min. Urine. Acidify 1 mL urine to pH 4 with 3 mg of citric acid, dilute with an equal volume MeCN, centrifuge at 18 000 g for 4 min.

HPLC VARIABLES
Column: 250 × 4.6 5μm Spherisorb Octyl
Mobile phase: MeOH:20 mM pH 3.6 potassium citrate + 0.02 mM tetramethylammonium hydrogen sulfate 48:52
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: metabolites, glucuronides

KEY WORDS
serum

REFERENCE
Hansen-Moller,J.; Dalgaard,L.; Hansen,S.H. Reversed-phase high-performance liquid chromatographic assay for the simultaneous determination of diflunisal and its glucuronides in serum and urine. Rearrangement of the 1-O-acylglucuronide, *J.Chromatogr.*, **1987**, *420*, 99–109.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablet and add amount containing ca. 50 mg diflunisal to 30 mL MeCN, sonicate, dilute to 50 mL, filter. Add 40 μL filtrate to 300 μL 1 mg/mL IS in MeCN, dilute to 5 mL with MeCN, inject 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:glacial acetic acid:THF 200:200:80:20
Flow rate: 1.8
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Retention time: 3.9

Internal standard: diflunisal ethyl ester (10.6)
Limit of detection: 5000 ng/mL

OTHER SUBSTANCES
Noninterfering: aspirin, phenacetin, salicylic acid
Interfering: diclofenac, indomethacin, ibuprofen

KEY WORDS
tablets

REFERENCE
Abdel-Hamid,M.E.; Najib,N.M.; Suleiman,M.S.; El-Sayed,Y.M. Differential spectrophotometric, fluorimetric and high-performance liquid chromatographic determination of diflunisal and its tablets, *Analyst*, **1987**, *112*, 1527–1530.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:10 mM sodium carbonate 18:82. B was MeCN:50 mM sodium carbonate 33:67. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: tolmetin, aspirin, ibuprofen, fenbufen, naproxen, carprofen, indomethacin

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 23.2

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone propionate, tranylcypromine, tripelennamine

Interfering: testosterone

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine,

pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.91 (A), 5.95 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyra-

zone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Allow frozen urine to thaw, vortex 10 s, allow to settle, dilute aliquot of clear supernatant 1:2 to 1:40 with 67 mM pH 5.75 phosphate buffer. Add 100 μL aliquot to 550 μL 67 mM pH 5.75 phosphate buffer + 100 μL 50 μg/mL naproxen in MeCN, inject 20 μL aliquot.

HPLC VARIABLES
Guard column: Whatman Co:Pell ODS
Column: Nova Pak C18
Mobile phase: Gradient. MeOH:buffer from 46:54 to 80:20 over 16 min (Buffer was 1.4 g/L Na_2HPO_4 adjusted to pH 2.5 with 85% phosphoric acid.)
Flow rate: 1.3
Injection volume: 10
Detector: F ex 258 em 428

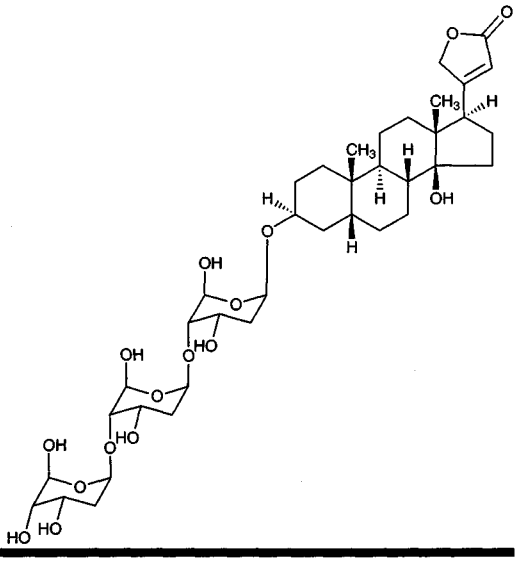
CHROMATOGRAM
Retention time: 16
Internal standard: naproxen (13)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Loewen,G.R.; Macdonald,J.I.; Verbeeck,R.K. High-performance liquid chromatographic method for the simultaneous quantitation of diflunisal and its glucuronide and sulfate conjugates in human urine, *J.Pharm.Sci.*, **1989**, *78*, 250–255.

Digitoxin

Molecular formula: $C_{41}H_{64}O_{13}$
Molecular weight: 764.95
CAS Registry No.: 71-63-6
Merck Index: 3206



SAMPLE

Matrix: blood

Sample preparation: Add 40 μL 1 μg/mL oleandrin in MeOH, 1 mL pH 9.5 ammonium chloride buffer, and 5 mL chloroform:2-propanol 95:5 (Caution! Chloroform is a carcinogen!) to 4 mL plasma, shake gently on a horizontal agitator for 10 min, centrifuge at 3500 g for 10 min, evaporate the lower organic phase to dryness in a rotary evaporator at 45°. Add 25 μL MeOH:2 mM pH 3.0 ammonium acetate buffer 20:80 to the dried residue, vortex for 10 s, centrifuge at 10000 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 1.0 5 μm Opti-Guard C18 (Interchim, France)
Column: 150 × 2.0 4 μm NovaPak C18
Mobile phase: Gradient. A was MeCN. B was 2 mM pH 3.0 ammonium acetate buffer. A: B from 20:80 to 38:62 in 5 min, from 38:62 to 65:35 in 1 min, from 65:35 to 70:30 in 2.5 min, maintain at 70:30 for 1 min, from70:30 to 20:80 in 0.5 min, maintain at 20:80 for 5 min (At the end of each chromatographic session rinse the column with MeCN:water 80: 20 for 30 min, then 50:50 for 3 h.)
Flow rate: 0.2
Injection volume: 5
Detector: MS, Perkin-Elmer Sciex API-100, ionspray, nebulizing nitrogen gas, positive ionization mode 4.5 kV, orifice +20 V, electron multiplier +2600 V, m/z 783

CHROMATOGRAM

Retention time: 8.08
Internal standard: oleandrin (8.30)
Limit of detection: 200 pg/mL

OTHER SUBSTANCES

Extracted: digoxin, lanatoside C, acetyldigitoxin

KEY WORDS

plasma

REFERENCE

Tracqui,A.; Kintz,P.; Ludes,B.; Mangin,P. High-performance liquid chromatography-ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma, *J.Chromatogr.B*, **1997**, *692*, 101–109.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 219.3

CHROMATOGRAM

Retention time: 18.725

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Ampoules. Add the contents of 1 ampoule (2 mL) to 15 mL 2% sodium bicarbonate solution, extract 5 times with 10 mL portions of chloroform:isopropanol 60:40, wash each extract with the same 10 mL portion of water, wash with another 10 mL portion of water. Combine the organic layers and evaporate them to dryness, transfer the residue to another tube with two 1 mL portions of chloroform:pyridine 10:1, evaporate to dryness under reduced pressure at 50°, add 200 μL reagent, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure at 50°, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject a 20 μL aliquot. Bulk. Prepare a solution in pyridine containing ≤10 mg/mL. Add 150 μL reagent to 50 μL solution, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure at 50°, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject a 20 μL aliquot. (Prepare reagent fresh each day by dissolving 100 mg 4-nitrobenzoyl chloride in 1 mL pyridine with gentle warming.)

HPLC VARIABLES
Column: 200 × 3 5 μm Merckosorb SI 60
Mobile phase: n-Hexane:chloroform:MeCN 30:10:9
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.2
Limit of detection: 11 ng/mL (100 μL injection)

OTHER SUBSTANCES
Simultaneous: diginatigenin, diginatin, digitoxigenin, digoxigenin, digoxin, gitaloxigenin, gitaloxin, gitoxigenin, gitoxin, lanatoside A, lanatoside B, lanatoside C, lanatoside D, lanatoside E

KEY WORDS
ampoules; normal phase; derivatization

REFERENCE
Nachtmann,F.; Spitzy,H.; Frei,R.W. Rapid and sensitive high-resolution procedure for digitalis glycoside analysis by derivatization liquid chromatography, *J.Chromatogr.*, **1976**, *122*, 293–303.

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. 1 mL Urine + 2 mL dichloromethane, shake by hand 4 times, centrifuge 1650 g. Remove organic layer and wash it twice with 2 mL 5% sodium bicarbonate solution, evaporate under nitrogen at 50°. Add 25 mg 4-dimethylaminopyridine and 10 μL 1-naphthoyl chloride, add 100 μL MeCN, vortex thoroughly, place in water bath at 50° for 1 h, centrifuge, evaporate at 50° under nitrogen. Add 2 mL 5% sodium bicarbonate solution, shake mechanically for 5 min, add 2 mL chloroform, shake by hand. Remove organic layer and wash it twice with 2 mL 5% sodium bicarbonate solution, wash three times with 0.05 M HCl containing 5% NaCl, evaporate chloroform, dissolve residue in mobile phase. Feces. Dilute 5:1 (v/w) with 5 μg/mL clindamycin in water to stop bacterial metabolism, homogenize with mechanical shaking for 15 min. 1 g Homogenate + 1 mL water, vortex 30 s, shake 15 min, centrifuge 1 h. Pour off supernatant and extract it with 2 mL dichloromethane. Wash the extract twice with 2 mL 5% sodium bicarbonate solution, evaporate under nitrogen at 50°. Add 25 mg 4-dimethylaminopyridine and 10 μL 1-naphthoyl chloride, add 100 μL MeCN, vortex thoroughly, place in water bath at 50° for 1 h, centrifuge, evaporate at 50° under nitrogen. Add 2 mL 5% sodium bicarbonate solution, shake mechanically for 5 min, add 2 mL chloroform, shake by hand. Remove organic layer and wash it twice with 2 mL 5% sodium bicarbonate solution, wash three times with 0.05 M HCl containing 5% NaCl, evaporate chloroform, dissolve residue in mobile phase.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere SI
Mobile phase: Hexane:dichloromethane:MeCN 6:1:1
Flow rate: 1.8-2
Injection volume: 20-175
Detector: F ex 217 em 340 cut-off filter (max 372 nm)

CHROMATOGRAM
Retention time: 8.1
Internal standard: digitoxin
Limit of detection: 5 ng/mL (urine) or 50 ng/g (feces)

OTHER SUBSTANCES
Simultaneous: digoxin, metabolites

KEY WORDS
normal phase; digitoxin is IS

REFERENCE
Shepard,T.A.; Hui,J.; Chandrasekaran,A.; Sams,R.A.; Reuning,R.H.; Robertson,L.W.; Caldwell,J.H.; Donnerberg,R.L. Digoxin and metabolites in urine and feces: a fluorescence derivatization-high performance liquid chromatographic technique, *J.Chromatogr.*, **1986**, *380*, 89–98.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets, weigh out amount equivalent to 0.1 mg digitoxin, add 500 μL MeOH:water 50:50, sonicate for 5 min, add 5 mL 12.6 μg/mL progesterone in acetone:EtOH 90:10, sonicate for 10 min, centrifuge at 1400 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL MeOH, inject a 0.5 μL aliquot.

HPLC VARIABLES
Column: 102 × 0.5 5 μm SC-01 ODS in a PTFE tube (Japan Spectroscopic)
Mobile phase: MeCN:MeOH:water 10:20:17
Flow rate: 0.008
Injection volume: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 18
Internal standard: progesterone (23)

OTHER SUBSTANCES
Simultaneous: digitoxigenin, digitoxigenin monodigitoxoside, digitoxigenin bisdigitoxoside

KEY WORDS
microbore; tablets

REFERENCE
Fujii,Y.; Ikeda,Y.; Yamazaki,M. Determination of cardiac glycosides in digitoxin tablets and deslanoside injections by micro-HPLC, *J.Chromatogr.Sci.*, **1990**, *28*, 288–291.

SAMPLE
Matrix: solutions
Sample preparation: Add 15 mg 3,5-dinitrobenzoyl chloride to 200 μL of a solution in dry pyridine, shake for 2 h, evaporate to dryness under a stream of nitrogen, reconstitute with 1.5 mL ethyl acetate. Wash this solution four times with 1 mL portions of 2.5 mg/mL 4-dimethylaminopyridine in 5% sodium bicarbonate solution, wash four times with 1 mL portions of 1% HCl, wash four times with 1 mL portions of water, evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject a 0.2 μL aliquot.

HPLC VARIABLES
Column: 150 × 0.5 5 μm SC-01 octadecylsilyl in a PTFE column (Japan Spectroscopic)
Mobile phase: MeCN:water 80:28
Flow rate: 0.008
Injection volume: 0.2
Detector: UV 230

CHROMATOGRAM
Retention time: 45
Internal standard: gitoxin (53)
Limit of detection: 0.6 ng
Limit of quantitation: 2 ng

OTHER SUBSTANCES
Simultaneous: digitoxigenin, digitoxigenin monodigitoxoside, 3-epidigitoxigenin

KEY WORDS
derivatization; microbore

REFERENCE
Fujii,Y.; Oguri,R.; Mitsuhashi,A.; Yamazaki,M. Micro HPLC separation of 3,5-dinitrobenzoyl derivatives of cardiac glycosides and their metabolites, *J.Chromatogr.Sci.*, **1983**, *21*, 495–499.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:water 37:63
Flow rate: 1.7

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: digoxin, gitoxigenin

REFERENCE
Supelco Catalog, **1993**, p. 502.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dex-

tromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10 μL aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 250 × 4.6 Deltabond C18 (Keystone)

Mobile phase: Gradient. MeCN:water from 10:90 to 45:55 over 8 min.

Flow rate: 1.3

Injection volume: 10

Detector: E, Dionex pulsed electrochemical detector, integrated amperometry mode, 1.4 mm gold working electrode with 0.005 inch gasket, E1 +0.07 V, t1 400 ms, E2 +0.70 V, t2 120 ms, E3 1.00 V, t3 300 ms, stainless steel counter electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 1 M NaOH pumped at 0.5 mL/min and the mixture flowed through a 500 μL reaction coil (Dionex) to the detector.

CHROMATOGRAM

Retention time: 12

Limit of detection: 400 ng/mL

OTHER SUBSTANCES

Simultaneous: digitoxigenin, digitoxigenin bisdigitoxoside, digitoxigenin monodigitoxoside, digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, digoxin

KEY WORDS

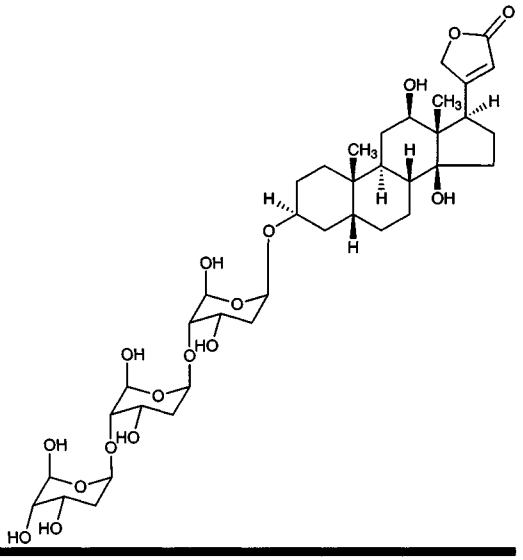
post-column reaction

REFERENCE

Kelly,K.L.; Kimball,B.A.; Johnston,J.J. Quantitation of digitoxin, digoxin, and their metabolites by high-performance liquid chromatography using pulsed amperometric detection, *J.Chromatogr.A*, **1995**, *711*, 289–295.

Digoxin

Molecular formula: $C_{41}H_{64}O_{14}$
Molecular weight: 780.95
CAS Registry No.: 20830-75-5
Merck Index: 3210



SAMPLE

Matrix: blood

Sample preparation: Add 40 μL 1 μg/mL oleandrin in MeOH, 1 mL pH 9.5 ammonium chloride buffer, and 5 mL chloroform:2-propanol 95:5 (Caution! Chloroform is a carcinogen!) to 4 mL plasma, shake gently on a horizontal agitator for 10 min, centrifuge at 3500 g for 10 min, evaporate the lower organic phase to dryness in a rotary evaporator at 45°. Add 25 μL MeOH:2 mM pH 3.0 ammonium acetate buffer 20:80 to the dried residue, vortex for 10 s, centrifuge at 10000 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 1.0 5 μm Opti-Guard C18 (Interchim, France)
Column: 150 × 2.0 4 μm NovaPak C18
Mobile phase: Gradient. A was MeCN. B was 2 mM pH 3.0 ammonium acetate buffer. A: B from 20:80 to 38:62 in 5 min, from 38:62 to 65:35 in 1 min, from 65:35 to 70:30 in 2.5 min, maintain at 70:30 for 1 min, from70:30 to 20:80 in 0.5 min, maintain at 20:80 for 5 min (At the end of each chromatographic session rinse the column with MeCN:water 80: 20 for 30 min, then 50:50 for 3 h.)
Flow rate: 0.2
Injection volume: 5
Detector: MS, Perkin-Elmer Sciex API-100, ionspray, nebulizing nitrogen gas, positive ionization mode 4.5 kV, orifice +20 V, electron multiplier +2600 V, m/z 799

CHROMATOGRAM

Retention time: 6.00
Internal standard: oleandrin (8.30)
Limit of detection: 250 pg/mL

OTHER SUBSTANCES

Extracted: digitoxin, lanatoside C, acetyldigitoxin

KEY WORDS

plasma

REFERENCE

Tracqui,A.; Kintz,P.; Ludes,B.; Mangin,P. High-performance liquid chromatography-ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma, *J.Chromatogr.B*, **1997**, *692*, 101–109.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE column by packing 200 mg LCA-SiO6 short-chain alkyl silica material (Sykam) into a 4 mL column, wash with 5 mL MeCN, wash with 4 mL water. 1 mL Plasma or serum + 100 μL (1 mL) water, add to the SPE cartridge, wash with 2 (1) mL MeOH:water 20:80, wash with 3 mL water, wash with 2 (1) mL acetone water 15:85, suck dry, wash with 1.5 (1) mL hexane:ethyl acetate 90:10, (wash with 2 mL hexane:acetone 90:10), dry with a stream of nitrogen, elute with 1.5 (0.5) mL hexane: acetone 60:40. Evaporate the eluate to dryness, reconstitute the residue in 300 μL MeOH: water 20:80, inject the whole amount. (Values in parentheses represent those given for the schematic procedure which differs from the written procedure.)

HPLC VARIABLES
Guard column: 25 × 4 40-63 μm LiChrolut RP18 (Merck)
Column: 100 × 2 4 μm Superspher RP18
Mobile phase: Gradient. MeCN:water 10:90 for 0.1 min, to 18:82 over 3.9 min, to 26:74 over 15 min, to 75:25 over 7 min, return to initial conditions over 3 min, re-equilibrate for 15 min.
Flow rate: 0.2
Injection volume: 300
Detector: F ex 379 em 423 following post-column reaction. The column effluent mixed with 0.08% copper(II) acetate in 95% sulfuric acid pumped at 0.35 mL/min and the mixture flowed through a knitted 5 m × 0.3 mm ID PTFE coil at 75° to the detector.

CHROMATOGRAM
Retention time: 21.1
Limit of detection: 70 pg
Limit of quantitation: 280 pg

OTHER SUBSTANCES
Extracted: digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside

KEY WORDS
post-column reaction; plasma; serum; SPE

REFERENCE
Belsner,K.; Büchele,B. Fluorescence detection of cardenolides in reversed-phase high-performance liquid chromatography after post-column derivatization, *J.Chromatogr.B*, **1996**, *682*, 95–107.

SAMPLE
Matrix: blood, perfusate
Sample preparation: 200 μL Plasma or perfusate + 20 μL ethinyl estradiol solution + 5 mL dichloromethane, vortex, centrifuge for 10 min. Remove a 4.5 mL aliquot of the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: LiChrocart 100 RP-18
Mobile phase: MeOH:isopropanol:dichloromethane:water 40:9:4:47
Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Internal standard: ethinyl estradiol (17α-ethynylestradiol)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE

Su,S.-F.; Huang,J.-D. Inhibition of the intestinal digoxin absorption and exsorption by quinidine, *Drug Metab.Dispos.*, **1996**, *24*, 142–147.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 220.5

CHROMATOGRAM

Retention time: 13.852

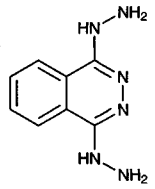
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Dihydralazine



Molecular formula: $C_8H_{10}N_6$
Molecular weight: 190.21
CAS Registry No.: 484-23-1, 7327-87-9 (sulfate)
Merck Index: 3212
Lednicer No.: 1 353

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 260

CHROMATOGRAM

Retention time: 3.70
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 219.3

CHROMATOGRAM

Retention time: 2.81

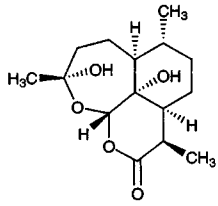
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Dihydroartemisinin



Molecular formula: $C_{15}H_{24}O_5$
Molecular weight: 284.36
Merck Index: 856

SAMPLE

Matrix: solutions
Sample preparation: Stir 1 μmole dihydroartemisinin, 1.5 μmoles dihydrofluorescein diacetate (diacetyldihydrofluorescein), 5 μmoles 4-dimethylaminopyridine, and 15 μmoles N,N'-dicyclohexylcarbodiimide in 750 μL dichloromethane at room temperature for 8 h (or at 40° for 4 h), inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: silica
Column: 100 × 8 Radial-PAK M-Porasil
Mobile phase: Hexane:isopropanol 95:5
Flow rate: 1.5
Injection volume: 10
Detector: UV 235

CHROMATOGRAM

Retention time: 5
Limit of detection: 0.1 ng

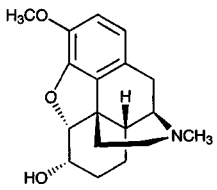
KEY WORDS

derivatization; normal phase

REFERENCE

Luo,X.-d.; Xie,M.; Zou,A.-q. Sub-nanogram detection of dihydroartemisinin after chemical derivatization with diacetyldihydrofluorescein followed by high-performance liquid chromatography and UV absorption, *Chromatographia*, **1987**, *23*, 112–114.

Dihydrocodeine



Molecular formula: $C_{18}H_{23}NO_3$
Molecular weight: 301.39
CAS Registry No.: 125-28-0
Merck Index: 3214
Lednicer No.: 1 288

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 10 mL MeOH, 5 mL MeCN:pH 2.4 phosphate buffer 20:80, and 10 mL water. 1 mL Plasma + 25 μL water + 25 μL 10 μg/mL N-ethylmorphine in water + 3 mL 0.5 M buffer, add to the SPE cartridge, wash with 20 mL 5 mM buffer, wash with 20 mL water, elute with 2 mL MeCN:pH 2.4 phosphate buffer 20:80, inject a 200 μL aliquot of the eluate. (The 0.5 M buffer was 33 g ammonium sulfate in 400 mL water, adjust the pH to 9.6 with 28% ammonia, make up to 500 mL with water. The 5 mM buffer was 0.6 g ammonium sulfate in 800 mL water, adjust pH to 9.6 with 28% ammonia, make up to 1 L with water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Crest-Pak C18S (Jasco)
Mobile phase: MeCN:buffer 25:75 (Buffer was 1.3 g sodium dodecylsulfate and 23.4 g $NaH_2PO_4.2H_2O$ in 2.5 L water, adjust pH to 2.4 with 85% phosphoric acid, make up to 3 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 200
Detector: E, ESA Coulochem 5010A, 5100A dual electrode analytical cell, first electrode +0.4 V (for IS), second electrode +0.8 V (for dihydrocodeine), 5021 conditioning cell between column output and analytical cell +0.15 V, 5020 guard cell at outlet from pump + 0.85 V

CHROMATOGRAM

Retention time: 21
Internal standard: N-ethylmorphine (15)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; dog; SPE

REFERENCE

Ohno,M.; Shiono,Y.; Konishi,M. Simultaneous determination of dihydrocodeine and its metabolites in dog plasma by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.B*, **1994**, *654*, 213–219.

SAMPLE

Matrix: blood, CSF
Sample preparation: Prepare 500 mg 3 mL Bond Elut C2 cartridges by rinsing with 2 mL MeOH then 2 mL 50 mM pH 7.5 Tris-HCl buffer. Apply 1 mL serum or CSF + 1 mL 50 mM pH 7.5 Tris-HCl buffer to the column and wash column with 10 mL 50 mM pH 7.5 Tris-HCl buffer. Elute with 2 mL 50% MeCN containing 0.1% trifluoroacetic acid. Freeze dry eluent or dry an aliquot at 40° under a stream of nitrogen, dissolve residue in

2560 μL mobile phase, inject 20-200 μL aliquot. (N.B. Extraction of morphine and codeine but not dihydrocodeine was demonstrated in the paper.)

HPLC VARIABLES
Guard column: Hexyl
Column: 150 × 4.6 Spherisorb S5 C6
Mobile phase: Gradient. A 0.1% trifluoroacetic acid in water; B 0.1% trifluoroacetic acid in 40% MeCN. 16% B for 2 min then to 50% B over 10 min then to 100% B over 2 min, after 7 min return to original conditions over 2 min.
Flow rate: 1
Injection volume: 20-200
Detector: F ex 280 em 335

CHROMATOGRAM
Retention time: 12
Limit of detection: 1.11 ng/mL

OTHER SUBSTANCES
Extracted: morphine, normorphine, codeine, metabolites
Simultaneous: diamorphine

KEY WORDS
serum; SPE

REFERENCE
Venn,R.F.; Michalkiewicz,A. Fast reliable assay for morphine and its metabolites using high-performance liquid chromatography and native fluorescence detection, *J.Chromatogr.*, **1990**, *525*, 379–388.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 4.7

KEY WORDS
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W

Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4.62

OTHER SUBSTANCES

Simultaneous: buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: mephentermine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: urine

Sample preparation: Condition a 300 mg Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL water. 5 mL Urine + 1 mL concentrated HCl, vortex, heat at 120° for 30 min, cool, adjust pH to between 7.0 and 8.0 with 10 M KOH. 5 mL Urine or hydrolysed urine + nalorphine, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL pH 4 acetate buffer, wash with 2 mL MeOH, elute with 2 mL dichloromethane:isopropanol 80:20 containing 2% ammonia. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 0.5-1 mL pentane:dichloromethane 90:10. (Use unhydrolysed urine to determine diamorphine and unconjugated compounds.)

HPLC VARIABLES

Column: 200 × 2 3 μm Hypersil

Mobile phase: Pentane:dichloromethane:MeOH containing 0.5% diethylamine 65:29.8:5.2
Flow rate: 0.4
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 13
Internal standard: nalorphine (5)
Limit of detection: <20 ng/mL

OTHER SUBSTANCES

Extracted: codeine, 6-monoacetylmorphine, pholcodine, diamorphine, morphine
Simultaneous: diphenhydramine, ephedrine, hydrocodone
Noninterfering: aspirin, caffeine, chlordiazepoxide, dextropropoxyphene, diazepam, lignocaine, naloxone, norcodeine, normorphine, papaverine, procaine, quinine, theobromine, theophylline

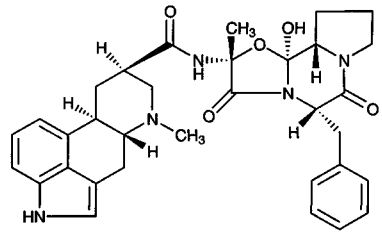
KEY WORDS

normal phase; SPE

REFERENCE

Low,A.S.; Taylor,R.B. Analysis of common opiates and heroin metabolites in urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 225–233.

Dihydroergotamine



Molecular formula: $C_{33}H_{37}N_5O_5$
Molecular weight: 583.69
CAS Registry No.: 511-12-6, 6190-39-2 (mesylate)
Merck Index: 3217

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 1 µg/mL dihydroergocristine mesylate in water + 30 µL 5 M NaOH + 7 mL chloroform, shake on a reciprocal shaker for 10 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase, inject a 10-30 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 µm RP-8 (Merck)
Mobile phase: MeCN:buffer 60:40 (Buffer was 9 mM NaH_2PO_4 and 9 mM Na_2HPO_4, pH 7.2.)
Flow rate: 1
Injection volume: 10-30
Detector: UV 223 or F ex 295 em 350

CHROMATOGRAM

Retention time: 5.2
Internal standard: dihydroergocristine mesylate (7.9)
Limit of detection: 0.5-0.7 ng/mL (F), 5-10 ng/mL (UV)

OTHER SUBSTANCES

Simultaneous: dihydroergocornine, dihydroergocryptine

KEY WORDS

plasma; rat

REFERENCE

Zecca,L.; Bonini,L.; Bareggi,S.R. Determination of dihydroergocristine and dihydroergotamine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1983**, *272*, 401–405.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 3 mL Plasma + 100 µL 75 ng/mL IS in water + 300 µL 25% ammonium hydroxide, vortex for 15 s, centrifuge at 1500 g for 5 min. Remove the supernatant and add it to a 3 mL Extrelut SPE cartridge, let stand for 15 min, elute with 15 mL diethyl ether. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute in 200 µL MeCN:ammonium carbamate 50:50, vortex for 15 s, sonicate for 30 s, centrifuge at 2000 g for 5 min, inject a 100 µL aliquot of the supernatant onto column A and start the gradient, after 4.5 min switch effluent from column A to column B for 1.4 min. Next, wash column A with MeCN for 1.5 min and A:B 60:40 for 2 min, stop flow through column B for this time. At the end of this time reconnect column B and elute with both columns with A:B 60:40, monitor the effluent from column B. At the end of the elution wash both columns with MeCN for 4.5 min, column B with A:B 60:40 for 5.5 min, and column A with A:B 100:0 for 3.5 min. Urine. Condition a Sep-Pak C18 SPE cartridge with 5 mL MeCN and 5 mL 10 mM ammonium carbamate. 5 mL Urine + 100 µL 250 ng/

mL IS in water + 400 μL 25% ammonium hydroxide, vortex for 15 s, centrifuge at 1500 g for 5 min. Remove the supernatant and add it to the SPE cartridge at 10 mL/min, wash with 1.5 mL MeCN:ammonium carbamate 45:55, apply vacuum to remove most of liquid, elute with 2 mL MeCN:MeOH 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute in 200 μL MeCN:ammonium carbamate 50:50, vortex for 15 s, sonicate for 30 s, centrifuge at 2000 g for 5 min, inject a 100 μL aliquot of the supernatant onto column A and start the gradient, after 4.5 min switch effluent from column A to column B for 1.4 min. Next, wash column A with MeCN for 1.5 min and A:B 60:40 for 2 min, stop flow through column B for this time. At the end of this time reconnect column B and elute with both columns with A:B 60:40, monitor the effluent from column B. At the end of the elution wash both columns with MeCN for 4.5 min, column B with A:B 60:40 for 5.5 min, and column A with A:B 100:0 for 3.5 min.

HPLC VARIABLES

Column: A 30 × 2.1 30 μm Aquapore RP 300 (Brownlee); B 83 × 4.6 3 μm HS3 C18 (Perkin-Elmer)
Mobile phase: Gradient. A was 10 mM pH 8.5 ammonium carbamate. B was MeCN. A:B 100:0 for 0.5 min, to 78:22 over 1.5 min, to 66:34 over 3 min, then as above.
Column temperature: 30
Flow rate: 1.8
Injection volume: 100
Detector: F ex 296 em 355

CHROMATOGRAM

Retention time: 13.3
Internal standard: dihydroergocornine methanesulfonate (14.5)
Limit of detection: 0.1 ng/mL (urine), 0.08 ng/mL (plasma)

KEY WORDS

plasma; SPE; column-switching; pharmacokinetics

REFERENCE

Humbert,H.; Denouel,J.; Chervet,J.P.; Lavene,D.; Kiechel,J.R. Determination of sub-nanogram amounts of dihydroergotamine in plasma and urine using liquid chromatography and fluorimetric detection with off-line and on-line solid-phase drug enrichment, *J.Chromatogr.*, **1987**, *417*, 319–329.

SAMPLE

Matrix: microsomal incubations
Sample preparation: 0.5 mL Microsomal incubation + 1 mL MeOH.

HPLC VARIABLES

Column: 250 × 3 μBondapak C18
Mobile phase: Gradient. MeCN:1 g/L ammonium carbonate from 0:100 to 60:30 over 65 min.
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 57

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver

REFERENCE

Delaforge,M.; Riviere,R.; Sartori,E.; Doignon,J.L.; Grognet,J.M. Metabolism of dihydroergotamine by a cytochrome P-450 similar to that involved in the metabolism of macrolide antibiotics, *Xenobiotica*, **1989**, *19*, 1285–1295.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

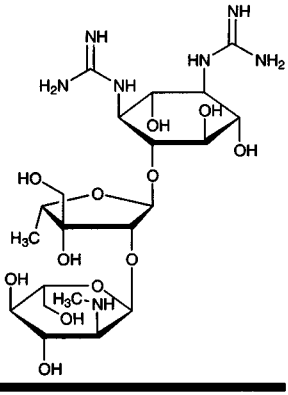
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluo-

perazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Dihydrostreptomycin



Molecular formula: $C_{21}H_{41}N_7O_{12}$
Molecular weight: 583.60
CAS Registry No.: 128-46-1, 5490-27-7 (sulfate)
Merck Index: 3222

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL MeOH and 20 mL water. 400 µL Serum + 2 mL buffer, add to the SPE cartridge, wash with 2 mL water, centrifuge at 2300 g for 5 min, elute with 5 mL MeOH. Concentrate the eluate to 200 µL under vacuum at 30°, add 200 µL mobile phase, inject a 100 µL aliquot. (Buffer was 50 mM sodium 1-hexanesulfonate and 25 mM Na_3PO_4, pH adjusted to 2.0 with phosphoric acid.)

HPLC VARIABLES

Guard column: 30 × 4.6 10 µm Spheri-10 RP-8 (Brownlee)
Column: 250 × 4 5 µm LiChrosorb RP-18
Mobile phase: MeCN:buffer 8:92 (Buffer was 3.76 g sodium 1-hexanesulfonate and 9.50 g $Na_3PO_4.12H_2O$ in 900 mL water, adjust pH to 3.0 with phosphoric acid, make up to 1 L with water.)
Column temperature: 55
Flow rate: 1
Injection volume: 100
Detector: UV 195

CHROMATOGRAM

Retention time: 20
Internal standard: dihydrostreptomycin

OTHER SUBSTANCES

Extracted: streptomycin

KEY WORDS

serum; SPE; dihydrostreptomycin is IS

REFERENCE

Kurosawa,N.; Kuribayashi,S.; Owada,E.; Ito,K.; Nioka,M.; Arakawa,M.; Fukuda,R. Determination of streptomycin in serum by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 379–385.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 4 mg/mL a solution in water, dilute a 3 mL aliquot to 50 mL with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeCN:buffer 8:92, pH 6.0 (Prepare by dissolving 3.8 g sodium 1-hexanesulfonate and 9.5 g Na_3PO_4 in 850 mL water and 80 mL MeCN, adjust pH to 6.0 with phosphoric acid, make up to 1 L with water. (Connect a 250 × 4.6 column of Bondapak C18/Corasil or Co:Pell ODS between pump and injector. Flush column with MeOH:water 50:50 at the end of the day.)
Column temperature: 25
Flow rate: 1.3
Injection volume: 25
Detector: UV 195

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: streptomycin, impurities

REFERENCE
Whall,T.J. Determination of streptomycin sulfate and dihydrostreptomycin sulfate by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *219*, 89–100.

SAMPLE
Matrix: milk
Sample preparation: Condition a Sep-Pak tC18 Vac (trifunctional) SPE cartridge with two 5 mL portions of MeOH, two 5 mL portions of water, three 5 mL portions of MeOH, three 5 mL portions of water, and 2 mL buffer. 6 mL Whole milk + 500 μL buffer + 1.5 mL 85% trichloroacetic acid in water, shake vigorously for 10 s, centrifuge at 4000 rpm for 3 min, add 2 mL dichloromethane, mix for 5 s, centrifuge at 4000 rpm for 5 min. Remove 6 mL of the supernatant and add it to 2.5 mL 4 M NaOH, mix for 2 s, centrifuge at 4000 rpm for 10 min. Remove the upper layer and add it to 2.5 mL 500 mM phosphoric acid, adjust pH to 6 ± 0.03 with 1 M NaOH or 500 mM phosphoric acid, add 1.5 mL buffer, mix, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeOH:water 3:97, wash with 16 mL MeOH:water 30:70 (gravity only), elute with 3.5 mL MeOH:formic acid 80:20 (under vacuum). Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue with 300 μL buffer, add 200 μL chloroform, mix vigorously for 10 s, centrifuge for 3 min, inject a 75 μL aliquot of the aqueous layer. (Prepare buffer by dissolving 4.45 g sodium 1-heptanesulfonate and 1.8 g $Na_2HPO_4.2H_2O$ in 750 mL water, adjust pH to 6.3 with 5 M phosphoric acid, make up to 1 L with water, adjust pH to 6.0 with 1 M phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:triethylamine:buffer 18.943:18:0.057:63 (Prepare buffer by dissolving 8.65 g sodium octanesulfonate and 4.68 g disodium 1,2-ethanedisulfonate in 750 mL water, adjust pH to 3.2 with acetic acid, add 0.891 g ninhydrin, make up to 1 L with water, adjust pH to 3.2.)
Flow rate: 1.4
Injection volume: 75
Detector: F ex 305 em 500 following post-column reaction. The column effluent mixed with 300 mM NaOH pumped at 0.5 mL/min in a 1.2 μL vortex mixer, the mixture was illuminated with UV light as it flowed through a 10 m × 0.3 mm i.d. PTFE coil at 80°, the effluent from the photochemical reactor was cooled to room temperature and passed to the detector.

CHROMATOGRAM
Retention time: 9
Limit of detection: 15 ng/mL
Limit of quantitation: 25 ng/mL

KEY WORDS
post-column reaction; post-column photochemical derivatization; SPE

REFERENCE
Hormazabal,V.; Yndestad,M. Determination of dihydrostreptomycin sulfate in milk by HPLC using ion-pair and postcolumn derivatization, *J.Liq.Chromatogr.*, **1995**, *18*, 2695–2702.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 μm RP-18
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)
Flow rate: 1.5
Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: neomycin, paromomycin, streptomycin

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut Certify II SPE cartridge with 3 mL MeCN, 1 mL water, and three 1 mL aliquots of buffer B, do not allow to dry. Homogenize (Ultra Turrax TP 18/10) 8 g Kidney or meat, 5 mL buffer A, and 1 mL 85% trichloroacetic acid in water for 6 s, centrifuge at 5000 rpm for 3 min, add 2 mL dichloromethane, mix for 6 s, centrifuge at 5000 rpm for 5 min. Remove a 7 mL aliquot of the supernatant and add it to 900 μL 4 M NaOH, blend, centrifuge at 4000 rpm for 5 min. Remove the upper layer and add it to 900 μL 500 mM phosphoric acid, adjust the pH to 5.5-5.8 with 1 M NaOH or 500 mM phosphoric acid, add 2.5 mL buffer B, add to the SPE cartridge at 1 mL/min, rinse out the tube with 1 mL buffer A, add the rinse to the SPE cartridge, wash with two 5 mL portions of buffer A, suck dry for 2 s after each wash, wash with three 5 mL portions of 25% ammonia, suck dry for 2 s after each wash, wash with two 1 mL portions of water, suck dry for 2 s after each wash, wash with 1 mL water, suck dry for 10 s, elute with two 1 mL portions of 20% formic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, mix for 3-4 s, evaporate to dryness, reconstitute with 400 μL buffer A, add 200 μL chloroform, mix vigorously for 10 s, centrifuge for 3 min. Filter (Costar Spin X with 0.22 μm nylon membrane) the aqueous layer while centrifuging at 5600 g for 2 min, inject a 25 μL aliquot of the filtrate. (Prepare buffer A by dissolving 4.45 g sodium 1-heptanesulfonate and 1.8 g Na_2HPO_4 in 750 mL water, adjust pH to 5.9 with 5 M phosphoric acid, adjust pH to 5.5 with 500 mM phosphoric acid, make up to 1 L with water, adjust pH to 5.5 with 500 mM phosphoric acid. Prepare buffer B by dissolving 13.35 g sodium 1-heptanesulfonate and 1.8 g Na_2HPO_4 in 750 mL water, adjust pH to 5.9 with 5

M phosphoric acid, adjust pH to 5.5 with 500 mM phosphoric acid, make up to 1 L with water, adjust pH to 5.5 with 500 mM phosphoric acid.)

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ + Plus
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ + Plus
Mobile phase: MeCN:buffer 32:68 (Prepare buffer by dissolving 8.65 g sodium octanesulfonate and 110 mg potassium 1,2-naphthoquinone-4-sulfonate in 750 mL water, make up to 1 L with water, filter (0.45 μm), adjust pH to 3.24 with 1 mL acetic acid.) (Streptomycin can also be determined with MeCN:buffer 30:70.)
Column temperature: 31
Flow rate: 0.6 for 0.5 min, 0.9 for 4 min, 0.6 for 9 min
Injection volume: 25
Detector: F ex 375 em 412 following post-column reaction. The column effluent mixed with 300 mM NaOH pumped at 0.3 mL/min in a 1.2 μL vortex mixer (Kratos PCRS 520) and the mixture flowed through a 15 m × 0.5 mm ID knitted coil at 40° and a room temperature heat exchanger to the detector.

CHROMATOGRAM

Retention time: 12
Limit of detection: 20 ppb
Limit of quantitation: 40 ppb

KEY WORDS

post-column reaction; muscle; kidney; SPE

REFERENCE

Hormazábal,V.; Yndestad,M. High performance liquid chromatographic determination of dihydrostreptomycin sulfate in kidney and meat using post column derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2259–2268.

SAMPLE

Matrix: tissue, milk
Sample preparation: Tissue. Condition a 5 mL 500 mg 40 μm Bakerbond aromatic sulfonic acid SPE cartridge with 5 mL water. Homogenize (Polytron Model PT 10/35) 5 g tissue with 10 mL 3.6% perchloric acid for 10-15 s, shake horizontally for 5 min, centrifuge at 2000 g for 5 min, add the supernatant to the SPE cartridge at 2 mL/min, wash with 3 mL water, allow to dry, elute with 9 mL buffer. Add 500 μL 200 mM 1-hexanesulfonic acid in water and 150 μL perchloric acid to the eluate, make up to 10 mL with water, filter (0.45 μm), inject a 2 mL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Milk. 10 g Milk + 3 mL 3.6% perchloric acid, shake horizontally for 30 s, centrifuge at 2000 g for 5 min. Add the supernatant to 70 μL 5 M NaOH and 500 μL 200 mM 1-hexanesulfonic acid, make up to 10 mL with water, filter (0.45 μm), inject a 2 mL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B (J. AOAC Int. 1994, 77, 765). (Prepare the buffer by dissolving 33.46 g K_2HPO_4 and 1.05 g KH_2PO_4 in 1 L water, pH 8.0. At the end of each day wash with MeCN:water 50:50 at 1.5 mL/min for 10 min.)

HPLC VARIABLES

Column: A 40 × 4.6 5 μm Inertsil C8; B 250 × 4.6 5 μm LC8-DB (Supelco)
Mobile phase: A 10 mM 1-Hexanesulfonic acid adjusted to pH 3.3 with acetic acid; B MeCN:water 17:83 containing 10 mM 1-hexanesulfonic acid and 400 μM 1,2-naphthoquinone-4-sulfonic acid, adjust pH to 3.3 with acetic acid
Flow rate: A 1; B 1.5

Injection volume: 2000
Detector: F ex 347 em 418 (tissue) or ex 365 em 418 (milk) following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.5 mL/min and the mixture flowed through a 2 mL reaction coil (Pickering, Mountain View CA) at 50° to the detector. (At the end of each day wash the post-column reaction system with 1% acetic acid for 10 min and with water for 10 min.)

CHROMATOGRAM

Retention time: 23
Limit of detection: 20 ppb

OTHER SUBSTANCES

Extracted: streptomycin

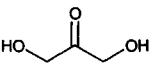
KEY WORDS

post-column reaction; column-switching; SPE; pig; cow; muscle; kidney

REFERENCE

Gerhardt,G.C.; Salisbury,C.D.C.; MacNeil,J.D. Determination of streptomycin and dihydrostreptomycin in animal tissue by on-line sample enrichment liquid chromatography, *J.AOAC Int.*, **1994**, *77*, 334–337.

Dihydroxyacetone



Molecular formula: $C_3H_6O_3$
Molecular weight: 90.08
CAS Registry No.: 96-26-4
Merck Index: 3225

SAMPLE
Matrix: formulations
Sample preparation: Lotions. Make up 10 mL lotion to 50 mL with water. 1 mL of this solution + 100 μL 650 μg/mL propyl paraben in EtOH:water 10:90, inject a 10 μL aliquot. Creams. 10 g cream + 50 mL MeCN:water 60:40, stir magnetically for 5 min, centrifuge an aliquot at 1500 rpm for 5 min. 1 mL of the supernatant + 100 μL 650 μg/mL propyl paraben in EtOH:water 10:90, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Hibar RP-8
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 2.5
Internal standard: propyl paraben (7.2)

KEY WORDS
lotions; creams

REFERENCE
Baruffini,A.; Caccialanza,G.; Gandini,C. Determinazione quantitiva del 1,3-hiidrossi-2-propanone (DHA) in preparazioni cosmetiche mediante HPLC [Quantitative determination of 1,3-dihydroxy-2-propanone (DHA) in cosmetics using HPLC], *Farmaco.[Prat].*, **1981**, *36*, 424–430.

SAMPLE
Matrix: formulations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 2 mL MeOH and 20 mL water. Dilute formulation ten-fold with water, add a 0.5 mL aliquot to the SPE cartridge, elute with three 1 mL portions of water, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 8 mm i.d. C18 radial compression (Waters)
Mobile phase: Water
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 3.8

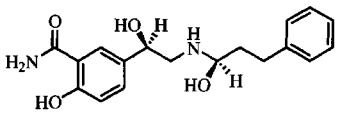
OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
SPE

REFERENCE

Bobin,M.F.; Martini,M.C.; Gudefin,A.; Cotte,J. Dosage de la dihydroxyacétone dans les émulsions [Assay of dihydroxyacetone in emulsions], *Farmaco.[Prat].*, **1983**, *38*, 403–414.

Dilevalol



Molecular formula: $C_{19}H_{24}N_2O_3$
Molecular weight: 328.41
CAS Registry No.: 75659-07-3, 75659-08-4 (HCl)
Merck Index: 3245
Lednicer No.: 4 20

SAMPLE

Matrix: bile, blood, urine
Sample preparation: 1 mL Plasma, blood, bile, or urine + 100 µL 1 µg/mL triamterene in MeOH + 1 (plasma, blood, urine) or 0.5 (bile) mL 100 mM sodium bicarbonate + 6 mL diethyl ether, shake horizontally for 10 min, centrifuge at 2500 g at 10° for 10 (plasma, blood, urine) or 20 (bile) min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 µL mobile phase, inject a 20 µL aliquot. (Hydrolyse conjugates as follows. 500 µL Plasma, blood, bile, or urine + 500 µL pH 5 buffer + 50 µL glusulase, heat at 50° for 24 h, add 50 µL 1 M NaOH, add 100 µL 1 µg/mL triamterene in MeOH, add 1 mL 100 mM sodium bicarbonate, proceed as above. In order to determine only the sulfate add 60 µL neutralized 100 mM 1,4-saccharolactone solution to inhibit β-glucuronidase.)

HPLC VARIABLES

Guard column: 25 × 2.3 5 µm PRP-1 (Hamilton)
Column: 150 × 4.1 5 µm PRP-1 (Hamilton)
Mobile phase: MeOH:buffer 65:35 (Buffer was 1 g sodium carbonate and 2.902 g sodium bicarbonate in 1 L water, pH 9.8.)
Flow rate: 0.5
Injection volume: 20
Detector: F ex 330 em 420

CHROMATOGRAM

Retention time: 4.4
Internal standard: triamterene (10)
Limit of detection: 10 ng/mL (urine), 20 ng/mL (bile), 1 ng/mL (plasma, blood)

OTHER SUBSTANCES

Noninterfering: acebutolol, brefanolol, carteolol, carvedilol, enalapril, furosemide, hydrochlorothiazide, isosorbide dinitrate, metoprolol, nifedipine, piretanide, propranolol, verapamil, xamoterol, xipamide

KEY WORDS

plasma; whole blood; pharmacokinetics

REFERENCE

Neubeck,M.; Becker,C.; Henke,D.; Rösch,W.; Spahn-Langguth,H.; Mutschler,E. Pharmacokinetics of dilevalol and its conjugates in man. Assay method for plasma, blood, urine and bile samples and preliminary pharmacokinetic studies, *Arzneimittelforschung*, **1993**, *43*, 953–957.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 25 µL 10 µg/mL IS in water + 500 µL 50 mM pH 9.0 Tris buffer + 4 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 5 min, freeze in dry ice/acetone. Add the organic layer to 200 µL 50 mM sulfuric acid, shake for 10 min, centrifuge at 2000 g for 5 min, discard the organic layer, heat the aqueous layer at 45° for 15 min, inject a 150 µL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 10 μm LiChroCART RP18 guard column
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:45 mM KH_2PO_4 25:75, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 2
Injection volume: 150
Detector: UV 216

CHROMATOGRAM

Retention time: 5.5
Internal standard: erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol(ICI 118 551) (14.3)
Limit of quantitation: 12.5 ng/mL

KEY WORDS

plasma; rat; human

REFERENCE

Dionisotti,S.; Bamonte,F.; Monopoli,A. High-performance liquid chromatographic assay of dilevalol, a β-adrenoceptor blocker, in rat and human plasma, *J.Chromatogr.*, **1990**, *530*, 458–462.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 18.71

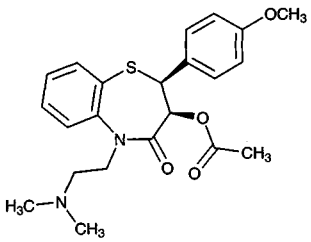
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Diltiazem



Molecular formula: $C_{22}H_{26}N_2O_4S$
Molecular weight: 414.53
CAS Registry No.: 42399-41-7, 33286-22-5 (HCl)
Merck Index: 3247
Lednicer No.: 3 198

SAMPLE

Matrix: blood
Sample preparation: 1.0 mL Plasma + 50 µL 5 mg/L desipramine HCl + 100 µL 50 mM pH 7.5 Na_2HPO_4/K_2HPO_4, vortex briefly. Add 5 mL diethyl ether and shake horizontally at 100 rpm for 20 min. Centrifuge at 3000 rpm for 10 min, snap-freeze in a dry-ice/EtOH bath. Add 100 µL 50 mM HCl to the decanted organic phase, vortex for 60 s, snap-freeze, decant organic phase to waste. Inject a 50 µL aliquot of the acid extract.

HPLC VARIABLES

Column: 100 × 4 5 µm Lichrocart RP-SelectB
Mobile phase: MeCN: 40 mM pH 5.5 Na_2HPO_4 buffer 25:75
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 215

CHROMATOGRAM

Retention time: 15.8
Internal standard: desipramine (18.9)
Limit of quantitation: 2.5 µg/L

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Morris,R.G.; Saccoia,N.C.; Jones,T.E. Modified liquid chromatographic assay for diltiazem and metabolites in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2385–2394.

SAMPLE

Matrix: blood
Sample preparation: Mix 400 µL serum with 20 µL 10 µg/mL IS in MeOH and 50 µL 10% sodium carbonate. Add 4 mL diisopropyl ether, shake vigorously for 4 min, centrifuge, freeze at -20°. Mix the organic layer with 100 µL 10 mM HCl, vortex carefully for 45 s using a microshaker, centrifuge, evaporate the aqueous phase to dryness under a stream of argon in a 56° water bath. Reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot. (Caution! Diisopropyl ether readily forms explosive peroxides!)

HPLC VARIABLES

Guard column: 20 × 4.6 5 µm Supelguard LC-CN
Column: 150 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:water:500 mM KH_2PO_4 36:62:2
Flow rate: 1.8
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 6.4
Internal standard: LU41616 (2'-[2-hydroxy-3-(3"-hydroxy-3"-methylbutylamino)propoxy]-3-phenylpropiophenone hydrochloride) (7.7)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, mexiletine, propafenone
Simultaneous: acebutolol, amiodarone, aprobarbital, atenolol, bupranolol, celiprolol, clobazam, debrisoquine, diazepam, flecainide, gallopamil, hexobarbiltal, lidocaine, mephenytion, metopolol, nadolol, pentobarbital, phenacetin, prazosin, procainamide, progesterone, propranolol, quinidine, sotalol, theophylline, verapamil

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High performance liquid chromatographic analysis of some antiarrhythmic drugs in human serum using cyanopropyl derivatized silica phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1169–1181.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize liver at 20 mg/mL in 50 mM pH 7.4 Tris-HCl buffer. 200 μL Plasma or 250 μL liver homogenate + 250 ng IS + pH 7.3 ammonium phosphate buffer + MTBE, extract. Remove the organic layer and add it to 250 μL 50 mM phosphoric acid, extract. Remove the aqueous layer and add it to 100 μL MeCN, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Chromegabond (ES Industries)
Mobile phase: MeCN:100 mM sodium perchlorate containing 50 mM phosphoric acid 34:66
Column temperature: 30
Flow rate: 1
Detector: UV 237

CHROMATOGRAM
Retention time: 4.5
Internal standard: N-methyl-N-ethyldiltiazem (9.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; liver; pharmacokinetics

REFERENCE
Los,L.E.; Welsh,D.A.; Herold,E.G.; Bagdon,W.J.; Zacchei,A.G. Gender differences in toxicokinetics, liver metabolism, and plasma esterase activity: Observations from a chronic (27-week) toxicity study of enalapril/diltiazem combinations in rats, *Drug Metab.Dispos.*, **1996**, *24*, 28–33.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove

the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.992

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve diltiazem hydrochloride in EtOH to give a diltiazem concentration of 130 μg/mL, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OD (Daicel Chemicals, Japan), (cellulose tris(3,5-dimethylphenyl carbamate) bonded to silica)
Mobile phase: n-Hexane:2-propanol:diethylamine 90:10:0.01
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10.5 (*d-cis*-isomer)

OTHER SUBSTANCES

Simultaneous: *l-trans*-isomer, *d-trans*-isomer, *l-cis*-isomer

KEY WORDS

chiral

REFERENCE

Yaku,K.; Aoe,K.; Nishimura,N.; Sato,T.; Morishita,F. Chiral resolution of four optical isomers of diltiazem hydrochloride on Chiralcel columns by packed-column supercritical fluid chromatography, *J.Chromatogr.A*, **1997**, *785*, 185–193.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve diltiazem hydrochloride in EtOH to give a diltiazem concentration of 1-5 mg/mL, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OD (Daicel Chemicals, Japan) (cellulose tris(3,5-dimethylphenyl carbamate) bonded to silica)

Mobile phase: Carbon dioxide:2-propanol:diethylamine 87:13:0.065

Column temperature: 50

Flow rate: 3

Injection volume: 5

Detector: UV 254 (connect a manual back-pressure regulator (Tescom) at 40° after the detector)

CHROMATOGRAM

Retention time: 7 (*d-cis*-isomer)

OTHER SUBSTANCES

Simultaneous: *l-trans*-isomer, *d-trans*-isomer, *l-cis*-isomer

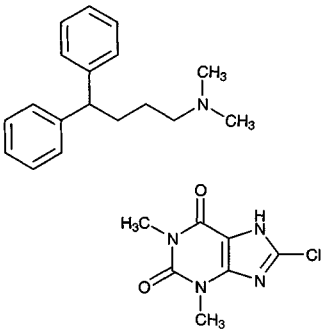
KEY WORDS

chiral; packed-column; SFC; pressure 180 kg/cm^2

REFERENCE

Yaku,K.; Aoe,K.; Nishimura,N.; Sato,T.; Morishita,F. Chiral resolution of four optical isomers of diltiazem hydrochloride on Chiralcel columns by packed-column supercritical fluid chromatography, *J.Chromatogr.A*, **1997**, *785*, 185–193.

Dimenhydrinate



Molecular formula: $C_{24}H_{28}ClN_5O_3$
Molecular weight: 469.97
CAS Registry No.: 523-87-5
Merck Index: 3252

SAMPLE

Matrix: blood, gum

Sample preparation: Serum. 3 mL Serum + 500 µL MeOH:100 mM NaH_2PO_4 10:90 + 150 µL concentrated perchloric acid, centrifuge at 10000 g for 20 min, inject a 500 µL aliquot of the supernatant. Gum. Freeze gum in liquid nitrogen for 30 min, pulverize, suspend in 5 mL 200 mM perchloric acid, shake for 30 min, centrifuge at 10000 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.5 10 µm Spherisorb S 10 ODS

Mobile phase: MeOH:buffer 40:60 (Buffer was 75 mM NaH_2PO_4, 750 mM EDTA (titriplex III), and 0.1% pentanesulfonic acid.)

Flow rate: 2

Injection volume: 500

Detector: UV 215

CHROMATOGRAM

Retention time: 4

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

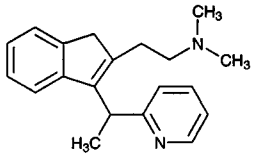
KEY WORDS

serum; pharmacokinetics

REFERENCE

Skofitsch,G.; Lembeck,F. Serum levels of dimenhydrinate. Determination by HPLC with UV detection after intake of dimenhydrinate in a coated chewing gum dragee, *Arzneimittelforschung*, **1983**, *33*, 1674–1676.

Dimethindene



Molecular formula: $C_{20}H_{24}N_2$
Molecular weight: 292.42
CAS Registry No.: 5636-83-9, 3614-69-5 (maleate)
Merck Index: 3265

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine,

rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 15.63

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: urine
Sample preparation: Dilute 1 mL human urine or 50-200 μL rat urine with 20 mM pH 4.6 sodium acetate to a final volume of 2 mL. Incubate with 1000 IU β-glucuronidase containing arylsulfatase in 500 μL 0.2% (m/m) NaCl solution at 37° for 24 h. Extract with two 3 mL portions of cyclohexane:ethyl acetate 1:1 for 10 min, centrifuge at 4000 rpm for 20 min, evaporate the organic layer under a stream of nitrogen, dissolve the residue in MeOH:50 mM pH 4.5 phosphate buffer 1:1, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4 7 μm LiChrosorb Diol (Merck)
Column: 250 × 4.6 10 μm Chiralpak AD (Baker)
Mobile phase: n-Hexane:EtOH 92:8 containing 0.1% diethylamine (B)
Flow rate: 0.7
Injection volume: 20
Detector: UV 259

CHROMATOGRAM

Retention time: 7.5 (S-(+)), 8.0 (R-(-))

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; chiral; rat; pharmacokinetics

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

SAMPLE
Matrix: urine
Sample preparation: Dilute 1 mL human urine or 50-200 µL rat urine with 20 mM pH 4.6 sodium acetate to a final volume of 2 mL. Incubate with 1000 IU β-glucuronidase containing arylsulfatase in 500 µL 0.2% (m/m) NaCl solution at 37° for 24 h. Extract with two 3 mL portions of cyclohexane:ethyl acetate 1:1 for 10 min, centrifuge at 4000 rpm for 20 min, evaporate the organic layer under a stream of nitrogen, dissolve the residue in MeOH:50 mM pH 4.5 phosphate buffer 1:1, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 7 µm LiChrosorb Diol (Merck) + 50 × 4.6 Chiralcel OD (Baker, Gross-Gerau, Germany)
Column: 250 × 4 10 µm Chiralcel OD (Baker, Gross-Gerau, Germany)
Mobile phase: n-Hexane:EtOH 94:6 containing 0.1% diethylamine
Flow rate: 0.6
Injection volume: 20
Detector: UV 259

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; chiral; rat; pharmacokinetics

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

SAMPLE
Matrix: urine
Sample preparation: Dilute 200 µL rat urine with 1.8 mL water. 2 mL Human urine or diluted rat urine + 50 µL 80 µg/mL IS in 50 mM pH 4.5 phosphate buffer:MeOH 50:50 + 500 µL 25% ammonia, extract twice with 3 mL portions of cyclohexane: ethyl acetate 50:50 for 10 min. Centrifuge at 4000 rpm for 20 min, remove the organic layer and evaporate it under a stream of nitrogen. Reconstitute the residue in MeOH:50 mM pH 4.5 phosphate buffer 50:50, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 RP Select B RP8 (Merck)
Column: 250 × 4 5 µm RP Select B RP8 (Merck)
Mobile phase: Gradient. A was MeOH. B was THF. C was 50 mM pH 4.5 phosphate buffer. A:B:C from 27:3:70 to 22:3:75 over 18.9 min, to 48:4:48 over 10 min.
Flow rate: 0.5 for 18.9 min then 0.45
Injection volume: 20
Detector: UV 259

CHROMATOGRAM
Internal standard: codeine

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

SAMPLE
Matrix: urine
Sample preparation: Dilute 200 μL rat urine with 1.8 mL double distilled water. Mix 2 mL human urine or diluted rat urine with 50 μL 80 μg/mL codeine in MeOH:50 mM,pH 4.5 phosphate buffer 1:1 and 500 μL 25% aqueous ammonia. Extract with two 3 mL portions of cyclohexane:ethyl acetate 1:1 for 10 min, centrifuge at 4000 rpm for 20 min, evaporate the organic layer under a stream of nitrogen. Dissolve the residue in MeOH: 50 mM pH 4.5 phosphate buffer 1:1, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 RP Select B RP8 (Merck)
Column: 250 × 4 5 μm RP Select B RP8 (Merck)
Mobile phase: Gradient. A was MeOH. B was THF. C was 50 mM pH 4.5 phosphate buffer. A:B:C from 27:3:70 to 22:3:75 over 18.9 min, to 48:4:48 (step gradient), maintain at 48:4: 48 for 19 min.
Flow rate: 0.5 for 18.9 min, 0.45 for 19 min
Injection volume: 20
Detector: UV 259

CHROMATOGRAM
Internal standard: codeine

OTHER SUBSTANCES
Extracted: metabolite

KEY WORDS
human; rat; pharmacokinetics

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

SAMPLE
Matrix: urine
Sample preparation: Dilute 200 μL rat urine with 1.8 mL water. 2 mL Human urine or diluted rat urine + 50 μL 80 μg/mL IS in 50 mM pH 4.5 phosphate buffer:MeOH 50:50 + 500 μL 25% ammonia, extract twice with 3 mL portions of cyclohexane: ethyl acetate 50:50 for 10 min. Centrifuge at 4000 rpm for 20 min, remove the organic layer and evaporate it under a stream of nitrogen. Reconstitute the residue in MeOH:50 mM pH 4.5 phosphate buffer 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 RP Select B RP8 (Merck)
Column: 250 × 4 5 μm RP Select B RP8 (Merck)
Mobile phase: MeCN:50 mM pH 4.5 ammonium acetate buffer 45:55
Flow rate: 0.6
Injection volume: 20

Detector: MS, Finnigan MAT TSQ 7000 APCI, vaporiser 90°, source 210°

CHROMATOGRAM
Internal standard: codeine

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 500 µL 25% ammonia, extract twice with 3 mL portions of n-hexane for 10 min. Centrifuge at 4000 rpm for 20 min, remove the organic layer and evaporate it under a stream of nitrogen. Reconstitute the residue in mobile phase. Inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 7 µm LiChrosorb Diol
Column: 250 × 4.6 10 µm Chiralpak AD (Baker, Gross-Gerau, Germany)
Mobile phase: n-Hexane:EtOH 92:8 containing 0.1% diethylamine
Flow rate: 0.7
Injection volume: 20
Detector: UV 259

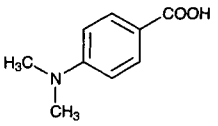
CHROMATOGRAM
Retention time: 7.5 (S-(+)), 8.5 (S-(-))

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
human; rat; chiral

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.

Dimethylaminobenzoic acid



Molecular formula: $C_9H_{11}NO_2$
Molecular weight: 165.19
CAS Registry No.: 619-84-1
Merck Index: 3282

SAMPLE

Matrix: blood, CSF
Sample preparation: 200 μL CSF or plasma + 200 μL MeOH, mix, add 1 mL buffer, add 7 mL dichloromethane:diethyl ether 40:60, shake for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness AT 45°, reconstitute the residue in 50 μl MeOH, vortex for 10 s, inject a 25 μL aliquot. (Buffer was 1 M KH_2PO_4 containing 0.2% ascorbic acid, pH adjusted to 4.2 with 1 M HCl.)

HPLC VARIABLES

Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 48:52
Flow rate: 1.5
Injection volume: 25
Detector: UV 215

CHROMATOGRAM

Retention time: 3.8
Internal standard: dimethylaminobenzoic acid

OTHER SUBSTANCES

Extracted: rifampin
Noninterfering: p-aminosalicylic acid, pyrazinamide, isoniazid

KEY WORDS

plasma; rabbit; dimethylaminobenzoic acid is IS

REFERENCE

Chan,K. Rifampicin concentrations in cerebrospinal fluid and plasma of the rabbit by high performance liquid chromatography, *Methods Find.Exp.Clin.Pharmacol.*, **1986**, *8*, 721–726.

SAMPLE

Matrix: sunscreen
Sample preparation: Weigh out 1 g sunscreen, add 2-10 mL mobile phase, stir magnetically for 5 min, filter (0.45 μm Millex-HV), inject an aliquot.

HPLC VARIABLES

Column: 200 × 5 5 μm Nucleosil C18
Mobile phase: MeCN:15 mM phosphoric acid 55:45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: benzocaine, p-aminobenzoic acid esters

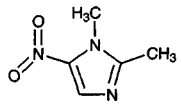
KEY WORDS

for amyl ester

REFERENCE

Bruze,M.; Gruvberger,B.; Thulin,I. PABA, benzocaine, and other PABA esters in sunscreens and after-sun products, *Photodermatol.Photoimmunol.Photomed.*, **1990**, *7*, 106–108.

Dimetridazole



Molecular formula: $C_5H_7N_3O_2$
Molecular weight: 141.13
CAS Registry No.: 551-92-8
Merck Index: 3315
Lednicer No.: 1 240

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 317.6

CHROMATOGRAM

Retention time: 9.955

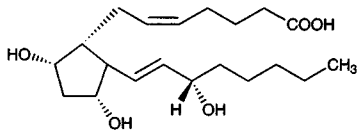
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Dinoprost



Molecular formula: $C_{20}H_{34}O_5$
Molecular weight: 354.49
CAS Registry No.: 551-11-1, 38562-01-5 (tromethamine salt)
Merck Index: 8065
Lednicer No.: 1 27

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Certify C18 SPE cartridge with water, MeCN, and 20 mM citric acid. Add 1 mL plasma to SPE cartridge, wash with 1 mL 20 mM citric acid, wash with 2 mL MeOH:water 10:90, wash with 2 mL cyclohexane, elute with 3 mL 3% ammonia in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeCN, add 200 μL 10 mM DBD-PZ in MeCN, add 300 μL 10 mM 2,2'-dipyridyl disulfide and 10 mM triphenylphosphine in MeCN, let stand at room temperature for 30 min, inject an aliquot. (DBD-PZ prepared from 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN added dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, extract three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ) as orange crystals, mp 121-2° (J. Chromatogr. 1991, 588, 61).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: Gradient. MeCN:water from 35:65 to 60:40 over 1 h
Column temperature: 40
Flow rate: 1
Detector: F ex 440 em 569

CHROMATOGRAM

Retention time: 25.7
Limit of detection: 1.7-5 fmole

OTHER SUBSTANCES

Extracted: alprostadil (prostaglandin E1), dinoprostone (prostaglandin E2), limaprost, 6-ketoprostaglandin F1α, prostaglandin F1α, prostaglandin D2, prostaglandin A1, prostaglandin B1

KEY WORDS

plasma; rat; SPE; derivatization

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Terao,T.; Imai,K. Sensitive fluorometric detection of prostaglandins by high performance liquid chromatography after precolumn labelling with 4-(*N,N*-dimethylaminosulphonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ), *Biomed.Chromatogr.*, **1992**, *6*, 143–148.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Serum + 44 μL MeOH + 1 μL pyridine, sonicate for 5 min, add 25 μL 100 mM reagent in DMF, add 20 μL 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, let stand at 25° for 2 h, centrifuge, inject an aliquot. (Reagent

was 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Pass dry hydrogen chloride into a mixture of 12.6 g methyl 2-furoate, 4.5 g paraformaldehyde, and 3.4 g anhydrous zinc chloride in 50 mL dry chloroform for 3 h while holding the reaction temperature at 30°. After cooling pour the contents of the flask into 100 mL cold water, remove the chloroform layer, extract the aqueous layer with chloroform (cf Coll. Czech. Chem. Commun. 1960, 25, 1058). Combine the chloroform layers, neutralize, dry over anhydrous calcium chloride, evaporate, distil to give 5-chloromethyl furyl-2-carboxylic acid methyl ester (bp 108°/4 mm Hg). Reflux 10 g 5-chloromethyl furyl-2-carboxylic acid methyl ester and 25 g silver carbonate in 100 mL THF: water 70:30 for 5 h, filter through Celite, concentrate the filtrate under reduced pressure, chromatograph the product on silica gel with chloroform to give 5-hydroxymethyl furyl-2-carboxylic acid methyl ester as a light yellow oil. Add a solution of 2.9 g 5-hydroxymethyl furyl-2-carboxylic acid methyl ester in 30 mL dichloromethane to 12 g pyridinium chlorochromate in 100 mL, dichloromethane, stir at room temperature for 4 h, evaporate to dryness under reduced pressure, chromatograph on silica with dichloromethane to give 5-formyl furyl-2-carboxylic acid methyl ester as a light yellow powder. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2- nitrophenyl)sulfide as yellow needles (mp 231-232°). Add 15 mL concentrated HCl dropwise to 1.5 g di(4,5-dimethoxy-2-nitrophenyl)sulfide and 4.5 g tin powder stirred at 40-50° in 150 mL EtOH, reflux for 1 h, cool to room temperature, filter, add 1.17 g 5-formyl furyl-2-carboxylic acid methyl ester to the filtrate, reflux for 1 h, cool, filter, chromatograph the solid on silica gel with dichloromethane, recrystallize from EtOH to give 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester as a yellow powder (mp 192-202°). Add 2 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 800 mg 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester in 20 mL EtOH, reflux for 30 min, collect the solid, wash with MeOH, dry under vacuum over phosphorus pentoxide to give 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a light yellow solid (mp 226-228°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 75:25 over 25 min, to 100:0 over 15 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 363 em 452

CHROMATOGRAM

Retention time: 41
Limit of detection: 50 fmole

OTHER SUBSTANCES

Extracted: alprostadil, arachidonic acid, dinoprostone, lauric acid, linoleic acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, prostaglandin $F_{1\alpha}$, stearic acid

KEY WORDS

derivatization

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *Anal.Sci.*, **1995**, *11*, 103–107.

SAMPLE

Matrix: blood, tissue

Sample preparation: Purify blood or tissue homogenate using a Sep-Pak C18 SPE cartridge, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeCN:water:acetic acid 45:55:0.01
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Extracted: dinoprostone, prostaglandin D2, 15-keto-prostaglandin E2, 13,14-dihydro-15-keto-prostaglandin F2α, 13,14-dihydro-15-keto-prostaglandin E2, 13,14-dihydro-15-keto-prostaglandin D2

KEY WORDS

rat; SPE

REFERENCE

Eguchi,N.; Kaneko,T.; Urade,Y.; Hayashi,H.; Hayaishi,O. Permeability of brain structures and other peripheral tissues to prostaglandins D_2, E_2 and $F_{2\alpha}$ in rats, *J.Pharmacol.Exp.Ther.*, **1992**, *262*, 1110–1120.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 5-50 mg compound in 1-2 mL MeCN, add a 3-fold molar excess p-nitrophenacyl bromide, add a 2-fold molar excess of N,N-diisopropylethylamine, let stand at room temperature for 15 min, dilute with 50 mL ethyl acetate, wash with 25 mL 200 mM pH 2.30 citrate buffer, wash with 200 mM pH 7.80 phosphate buffer, wash with 25 mL water, dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 45°, prepare a solution in dichloromethane:chloroform 50:50, inject an aliquot.

HPLC VARIABLES

Column: two 250 × 2.1 Zorbax-Sil columns in series
Mobile phase: Dichloromethane:hexane:MeOH 55:45:5
Flow rate: 0.3
Detector: UV 254

CHROMATOGRAM

Retention time: 10.95

OTHER SUBSTANCES

Simultaneous: dinoprostone

KEY WORDS

derivatization; normal phase

REFERENCE

Morozowich,W.; Douglas,S.L. Resolution of prostaglandin p-nitrophenacyl esters by liquid chromatography and conditions for rapid, quantitative p-nitrophenacylation, *Prostaglandins*, **1975**, *10*, 19–40.

SAMPLE

Matrix: bulk

Sample preparation: Add 10 mg prostaglandin to 1 mL 15 mg/mL 2-bromo-4'-nitroacetophenone in MeCN, add 5 μL N,N-diisopropylethylamine, mix, let stand at room temperature for at least 2 h. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL chloroform, add 500 μL 200 mg/mL silver nitrate in water, mix thoroughly, centrifuge. Filter (0.2 μm) the chloroform layer and inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil SCX impregnated with silver ion (Prepare the column by pumping 80 mL 1 M silver nitrate in water through the column, wash the column with water until a negative test for silver ion is obtained, wash with 50 mL EtOH, wash with 50 mL acetone, wash with 50 mL ethyl acetate, wash with 50 mL trichloroethane, and wash with 50 mL hexane.)

Mobile phase: Dioxane:MeCN 99.94:0.06 (Caution! Dioxane is a carcinogen!)

Detector: UV 254

CHROMATOGRAM

Retention time: k' 5.8

OTHER SUBSTANCES

Simultaneous: degradation products, alprostadil, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization

REFERENCE

Merritt,M.V.; Bronson,G.E. High-performance liquid chromatography of p-nitrophenacyl esters of selected prostaglandins on silver ion-loaded microparticulate cation-exchange resin, *Anal.Biochem.*, **1977**, *80*, 392–400.

SAMPLE

Matrix: bulk

Sample preparation: Mix 50-200 μg prostaglandins with 1 mL dry dichloromethane, 1 mg p-aminophenol, and 150 μL triethylamine, sonicate at 25° under nitrogen until dissolved, add at least a 3-fold molar excess of 2-bromo-1-methylpyridinium iodide, sonicate at 25° until the solution becomes cloudy, concentrate under a stream of nitrogen, add 1 mL 100 mM HCl, add 1 mL ethyl acetate, shake vigorously, centrifuge at 2500 rpm for 5 min, inject an aliquot of the supernatant. (Prepare 2-bromo-1-methylpyridinium iodide by analogy with the preparation of 2-chloro-1-methylpyridinium iodide. Add 15 g methyl iodide to 13.9 g 2-bromopyridine in 3 mL acetone at 0°, stir at room temperature for 3 days. Filter the precipitate and wash it with 50 mL dry ether, dry under reduced pressure to give 2-bromo-1-methylpyridinium iodide (Bull. Chem. Soc. Japan 1977, 50, 1863).)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C-18

Mobile phase: MeOH:water:perchloric acid 60:40:0.1 containing 50 mM sodium perchlorate

Column temperature: 25 ± 0.1

Flow rate: 1.1

Detector: E, 0.7 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 9

Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: dinoprostone

KEY WORDS
derivatization; guinea pig; plasma; human

REFERENCE
Ikenoya,S.; Hiroshima,O.; Ohmae,M.; Kawabe,K. Electrochemical detector for high performance liquid chromatography. IV. Analysis of fatty acids, bile acids and prostaglandins by derivatization to an electrochemically active form, *Chem.Pharm.Bull.(Tokyo)*, **1980**, *28*, 2941–2947.

SAMPLE
Matrix: cell suspensions

Sample preparation: Condition a 1 mL Bond Elut 5 μm ODS SPE cartridge with 2 mL diethyl ether, 2 mL MeOH, and 2 mL buffer. Condition a 1 mL 5 μm Bond Elut silica SPE cartridge with 2 mL MeOH:MeCN 15:85, with 2 mL acetone, and with 2 mL chloroform. Centrifuge at 4° at 12000 g for 10 min, acidify to pH 5.5 with 1% acetic acid, add to the ODS SPE cartridge, wash with 2 mL buffer, wash with 2 mL MeOH:buffer (pH 5.5), suck dry under vacuum for 2 min, wash with 2 mL petroleum ether, elute with 2 mL diethyl ether. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 20 μL MeOH, add 20 μL 1 mg/mL 9-anthroyldiazomethane in diethyl ether, heat at 37° for 6 h, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL chloroform, add to the silica SPE cartridge, wash with 3 mL chloroform, elute with 2 mL MeCN:MeOH 85:15. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, add 50 μL water, inject an aliquot. (Buffer was 0.0001% acetic acid adjusted to pH 5.5 with 1% NaOH. Synthesis of 9-anthryldiazomethane is as follows. Stir 10 g 9-anthraldehyde and 10 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 4 h, filter off the solid 9-anthraldehyde hydrazone and dry under vacuum. Dissolve 1 g 9-anthraldehyde hydrazone in 100 mL anhydrous ether, add 1 g activated manganese dioxide, add 500 μL saturated KOH in EtOH, stir for 3 h, filter off the manganese, evaporate the filtrate to give 9-anthryldiazomethane. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil ODS

Mobile phase: MeCN:water:acetic acid 62:37.9:0.1, pH adjusted to 5.5 with sodium acetate

Flow rate: 1

Detector: F ex 367 em 413

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Extracted: dinoprostone, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, thromboxane B_2

KEY WORDS
SPE; derivatization

REFERENCE
Wessel,K.; Kaever,V.; Resch,K. Measurement of prostaglandins from biological samples in the subnanogram range by fluorescence labelling and HPLC separation, *J.Liq.Chromatogr.*, **1988**, *11*, 1273–1292.

SAMPLE

Matrix: enzyme incubations

Sample preparation: Add 500 μL enzyme incubation to 1 mL MeOH, mix, add 4 mL 100 mM citric acid, add 500 mg anhydrous sodium sulfate, extract twice (alprostadil, dinoprostone) or 3 times (dinoprost) with 5 mL portions of dichloromethane. Pass the extracts through 1 g anhydrous sodium sulfate and evaporate them to dryness, reconstitute with 1 mL anhydrous MeCN containing a 3-fold molar excess of α,p-dibromoacetophenone, add 2 μL diisopropylethylamine, let stand for 1 h, evaporate to dryness, reconstitute with 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 11
Limit of quantitation: 5 μM

OTHER SUBSTANCES

Extracted: metabolites, alprostadil, dinoprostone

KEY WORDS

derivatization

REFERENCE

Fitzpatrick,F.A. High-performance liquid chromatographic analysis of prostaglandins formed during in vitro incubations with prostaglandin 15-dehydrogenase, *J.Pharm.Sci.*, **1976**, *65*, 1609–1613.

SAMPLE

Matrix: enzyme incubations

Sample preparation: 2 mL Enzyme incubation + 2 mL MeOH, centrifuge. Remove the supernatant and add it to 2 mL 100 mM citric acid and 500 mg anhydrous sodium sulfate, extract twice with 5 mL portions of dichloromethane. Dry the extracts over anhydrous sodium sulfate and evaporate them to dryness under a stream of nitrogen, reconstitute with 100-200 μL 1.2 mg/mL p-bromophenacyl bromide in MeCN, add 0.5 μL diisopropylethylamine, let stand at room temperature for 1 h, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Limit of quantitation: <3 μg

OTHER SUBSTANCES

Extracted: dinoprostone, prostaglandin A2, prostaglandin B2, prostaglandin D2, 15-methylprostaglandin B2

KEY WORDS

derivatization

REFERENCE
Fitzpatrick,F.A. High performance liquid chromatographic determination of prostaglandins F2α, E2, and D2 from in vitro enzyme incubations, *Anal.Chem.*, **1976**, *48*, 499–502.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with acetone, add hexanoic acid, heptanoic acid, and octanoic acid, add mixture to 5 g of a powdered 1:1 mixture of sodium sulfate and potassium bicarbonate + 2.7 mg dibenzo-18-crown-6 + 2.7 mg 4-bromomethyl-7-methoxycoumarin, let stand in the dark at 37° for 6 h, inject an aliquot.

HPLC VARIABLES
Column: 1060 × 0.2 3 μm Micro-Pak SP-18 (Varian)
Mobile phase: MeOH:MeCN:water 47.6:23.8:28.6
Column temperature: 35
Flow rate: 0.0006
Injection volume: 0.011
Detector: F ex 325 em 430 (laser-fluorescence)

CHROMATOGRAM
Retention time: 157
Internal standard: Hexanoic acid, heptanoic acid, octanoic acid
Limit of detection: 40-125 fmole

OTHER SUBSTANCES
Extracted: alprostadil (prostaglandin E1), prostaglandin D2, dinoprostone (prostaglandin E2), prostaglandin F1α, prostaglandin A2, prostaglandin B2, prostaglandin B1, prostaglandin A1

KEY WORDS
capillary; laser-fluorescence; derivatization

REFERENCE
McGuffin,V.L.; Zare,R.N. Femtomole analysis of prostaglandin pharmaceuticals, *Proc.Nat.Acad.Sci.USA*, **1985**, *82*, 8315–8319.

SAMPLE
Matrix: gastric mucosa
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL EtOH and 100 mL water. Homogenize gastric mucosa from three rats with 6 mL EtOH:50 mM pH 3.15 triethylammonium formate buffer 15:85 (cold), centrifuge at 300000 g for 15 min, resuspend the pellet in 3 mL EtOH:50 mM pH 3.15 triethylammonium formate buffer 15:85, centrifuge at 300000 g for 15 min. Combine the supernatants and add them to the SPE cartridge, wash with 50 mM pH 3.15 triethylammonium formate buffer 15:85, wash with 10 mL petroleum ether, elute with 10 mL methyl formate, store at -40°. Evaporate under helium, dissolve the residue in 100 μL diethyl ether with 2-3 drops MeOH, add 500 μL of a fresh solution of diazomethane, let stand in the dark at room temperature for 30 min, store in MeCN at -40°. (Prepare diazomethane from 1-methyl-3-nitro-1-nitrosoguanidine (Anal.Chem. 1973, 45, 2302).)

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was 100 mM ammonium acetate adjusted to pH 3.5 with formic acid. B was MeCN:200 mM ammonium acetate buffer 2:1. A:B from 65:35 to 15:85 over 27 min.
Injection volume: 20

Detector: MS, Hewlett-Packard 5988A, thermospray, insertion probe 180-185°, SIM m/z 386

CHROMATOGRAM
Retention time: 24.8
Limit of detection: 230 pg

OTHER SUBSTANCES
Extracted: dinoprostone, prostaglandin D2, 6-keto-prostaglandin F1α

KEY WORDS
rat; SPE; derivatization; LC-MS

REFERENCE
Abián,J.; Bulbena,O.; Gelpí,E. Thermospray liquid chromatography/mass spectrometry of prostaglandin methyl ester derivatives: application to the determination of prostaglandins E_2 and D_2 in rat gastric mucosa, *Biomed.Environ.Mass.Spectrom.*, **1988**, *16*, 215–219.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Acidify 1 mL microsomal incubation, to pH 3 with 1 M HCl, add to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, wash with 5 mL EtOH:water 15:85, wash with 5 mL petroleum ether, elute with 5 mL methyl formate. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute in 50 μL MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 330 × 4.6 Pecosphere 3X3C C18
Mobile phase: MeOH:water:acetic acid 50:50:0.01
Flow rate: 1.3
Injection volume: 5
Detector: radioactivity

CHROMATOGRAM
Retention time: 12.5

KEY WORDS
rat; liver; SPE

REFERENCE
Diczfalusy,U.; Alexson,S.E.H. Peroxisomal chain-shortening of prostaglandin $F_{2\alpha}$, *J.Lipid Res.*, **1988**, *29*, 1629–1636.

SAMPLE
Matrix: seminal fluid
Sample preparation: 1-5 μL Seminal fluid + 100 μL 5 μM IS in MeOH, mix, add 3 mL water to the supernatant, acidify to pH 3-4 with 100 mM HCl, extract with 7 mL ethyl acetate. Remove the ethyl acetate layer and evaporate it to dryness, reconstitute with MeOH. Evaporate to dryness in a clean tube, add 10 mg finely-powdered potassium bicarbonate:sodium sulfate 50:50, add 50 μL 0.4-1 mM 4-bromomethyl-7-acetoxycoumarin in acetone, add 50 μL 200 μM dibenzo-18-crown-6 in acetone, heat in the dark at 80° for 1 h, cool, inject a 20-40 μL aliquot. (Prepare 4-bromomethyl-7-acetoxycoumarin as follows. Reflux 50 g 7-hydroxy-4-methylcoumarin (β-methylumbelliferone) and 100 mL acetic anhydride for 1 h, cool, pour into 500 mL cold water, filter, dry the solid, recrystallize from EtOH to give 4-methyl-7-acetoxycoumarin. Reflux 10 g 4-methyl-7-acetoxycoumarin, 9 g N-bromosuccinimide, a little 2,2'-(azobis(2-methylpropionitrile) (α,α'-azobisisobutyronitrile, Eastman), and 100 mL carbon tetrachloride for 20 h, cool, evaporate under reduced

pressure to remove the solvent, wash the residue with water, filter, dry, recrystallize from ethyl acetate/cyclohexane to give 4-bromomethyl-7-acetoxycoumarin (mp 184-185°) (J. Chromatogr. 1982, 234, 121).)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:water from 30:70 to 90:10 over 99 min (Concave 1 curve (64 min) using a Japan Spectroscopic Model GP-A30 solvent programmer).
Column temperature: 50
Flow rate: 1
Injection volume: 20-40
Detector: F ex 365 em 460 following post-column reaction. The effluent from the column mixed with 100 mM NaOH pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 50° to the detector. (The prostaglandins are chromatographed as the coumarin derivatives then hydrolyzed in the post-column reactor to fluorescent 7-hydroxy-4-hydroxymethylcoumarin.)

CHROMATOGRAM

Retention time: 43
Internal standard: 16-methylprostaglandin $F_{1\alpha}$ (49)
Limit of detection: 10 fmole

OTHER SUBSTANCES

Extracted: dinoprostone, alprostadil

KEY WORDS

derivatization; post-column reaction

REFERENCE

Tsuchiya,H.; Hayashi,T.; Naruse,H.; Takagi,N. Sensitive high-performance liquid chromatographic method for prostaglandins using a fluorescence reagent, 4-bromomethyl-7-acetoxycoumarin, *J.Chromatogr.*, **1982**, *231*, 247–254.

SAMPLE

Matrix: seminal fluid
Sample preparation: Mix 50 μL seminal fluid with 500 μL dilute HCl (pH 3.0) and 500 μL ethyl acetate, vortex. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL water, add to a Toyopak-ODS SPE cartridge, elute with 200 μL MeOH. 100 μL Eluate + 100 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water + 100 μL 1% aqueous pyridine + 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran is as follows. Slowly add 153 g freshly distilled phosphorus oxychloride to 73 g anhydrous DMF with stirring at room temperature, add 125 g sesamol in portions over 4 h, stir at room temperature overnight, pour into ice water, filter. Dissolve the solid in ether and wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as slightly-yellow crystals (mp 125-126°). Pass HCl gas into 15.6 g ethyl 2-thiophenecarboxylate, 4.5 g paraformaldehyde, and 3.4 g zinc chloride in 50 mL chloroform with stirring at 30° over 4 h. Pour into ice water and extract with 50 mL chloroform. Wash the chloroform layer 3 times with water, wash twice with aqueous sodium bicarbonate solution, dry over anhydrous sodium sulfate, evaporate to remove the solvent, distil at 86-94°/0.15 mm Hg to yield ethyl 5-chloromethyl thiophene-2-carboxylate as a colorless oil. Heat 3 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 3.68 g ethyl 5-chloromethyl thiophene-2-carboxylate, and 2.49 g potassium carbonate in 100 mL anhydrous DMF at 110° for 16 h, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with chloroform, recrystallize from chloroform:hexane 25:75 to give 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as yellow crystals (mp

124-126°). Heat 1.5 g 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 1.2 g hydrazine hydrate in 15 mL DMF at 70° for 1 h (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 10 g hydrazine hydrate, add 20 mL water, filter. Wash the solid with MeOH and dry it under reduced pressure to give 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as a yellow powder (mp 262-263°).)

HPLC VARIABLES

Column: 250 × 4 5 μm Wakosil ODS-II 5C18 HG
Mobile phase: MeCN:water 34:66
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 373 em 483

CHROMATOGRAM

Retention time: 95
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Extracted: alprostadil, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization; SPE

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Yakata,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran as novel fluorescence derivatization reagents for carboxylic acids in liquid chromatography, *Anal.Chim.Acta*, **1995**, *300*, 243–251.

SAMPLE

Matrix: solutions
Sample preparation: Condition a Sep-Pak silica SPE cartridge with 5 mL THF:water 95:5, 5 mL MeCN, and 5 mL dichloromethane. Prepare a solution in EtOH, add a 500 ng/mL solution of panacyl bromide in THF:MeCN 20:80, for each 1 mL of reaction mixture add 3 μL triethylamine, heat at 37° for 3 h, add 0.5 mL of the reaction mixture to the SPE cartridge, wash with 10 mL dichloromethane, elute with 3 mL MeCN:MeOH 85:15, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in MeCN, inject an aliquot. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°) (Anal.Biochem. 1987, 165, 220).)

HPLC VARIABLES
Column: 300 × 3.9 fatty acid analysis column (Waters?)
Mobile phase: Gradient. MeCN:water:acetic acid from 56:44:0.1 to 65:35:0.1 over 15 min
Flow rate: 1.2
Detector: F ex 249 em 413 (cut-off filter) or UV 254

CHROMATOGRAM
Retention time: 22.78
Limit of detection: 280 pg (UV), 50 pg (F)

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin D2, 6-keto-prostaglandin F1α, 6-keto-prostaglandin E1, 13,14-dihydro-15-keto-prostaglandin F2α

KEY WORDS
derivatization; SPE

REFERENCE
Watkins,W.D.; Peterson,M.B. Fluorescent/ultraviolet absorbing ester derivative formation and analysis of eicosanoids by high-pressure liquid chromatography, *Anal.Biochem.*, **1982**, *125*, 30–40.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in 10 mM NaOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm PRP-1 styrene-divinylbenzene copolymer (Hamilton)
Mobile phase: MeCN:10 mM NaOH 21:79, measured pH 12.3 (At the end of each day wash the column with 40 mL water followed by 20 mL MeCN.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 206

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin A2, prostaglandin B2, prostaglandin D2, prostaglandin I2, thromboxane B2, 6-keto-prostaglandin F1α, 6-keto-prostaglandin E1

REFERENCE
Skrinska,V.; Thomas,G. High-performance liquid chromatography of prostacyclin, *J.Chromatogr.*, **1983**, *277*, 287–291.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, inject a 1 μL aliquot. Alternatively, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Column: 250 × 1.3 8 μm C18 (Chrompack)
Mobile phase: MeCN:10 mM pH 2.7 phosphoric acid 38:62

Flow rate: 0.06
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 20
Limit of detection: 0.14 pmole

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization; microbore

REFERENCE
Doehl,J.; Greibrokk,T. High-performance liquid chromatographic separation and determination of prostaglandins, oxidized by pyridinium dichromate. Optimization and applications, *J.Chromatogr.*, **1985**, *349*, 431–438.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of a solution in MeOH with 50 μL purified 9-anthryldiazomethane reagent, after 6 h inject an aliquot. (Purify 9-anthryldiazomethane on a 500 × 7.2 7 μm PG-pak C polystyrene gel column with ethyl acetate at 1 mL/min and UV 350 detection, inject 1 mg, collect the effluent when the purified compound elutes (20-22 min) and use it within 6 h.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm PG-Pak B silica gel
Mobile phase: Gradient. Isooctane:ethyl acetate:EtOH:acetic acid 90:10:0:1 for 15 min then 80:15:4:2 for 20 min (step gradient).
Flow rate: 1.2
Detector: F ex 365 em 412

CHROMATOGRAM
Retention time: 38
Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprostone, HHT, hydroxyeicosatetraenoic acid, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, prostaglandin $F_{1\alpha}$, thromboxane B_2

KEY WORDS
derivatization; normal phase

REFERENCE
Yamauchi,Y.; Tomita,T.; Senda,M.; Hirai,A.; Terano,T.; Tamura,Y.; Yoshida,S. High-performance liquid chromatographic analysis of arachidonic acid metabolites by pre-column derivatization using 9-anthryldiazomethane, *J.Chromatogr.*, **1986**, *357*, 199–205.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 5 μm cyano (IBM)
Mobile phase: Gradient. Hexane:isopropanol 98:2 for 12 min, then to 80:20 over 10 min, maintain at 80:20

Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: k' 9.63

OTHER SUBSTANCES
Extracted: arachidonic acid, prostaglandin H2, alprostadil (prostaglandin E1), prostaglandin D2, dinoprostone (prostaglandin E2), prostaglandin F1α, 6-ketoprostaglandin E1, 6-ketoprostaglandin F1α, thromboxane B2

REFERENCE
Zulak,I.M.; Puttemans,M.L.; Schilling,A.B.; Hall,E.R.; Venton,D.L. A fast, nondestructive purification scheme for prostaglandin H2 using a nonaqueous, bonded-phase high-performance liquid chromatography system, *Anal.Biochem.*, **1986**, *154*, 152–161.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compound in 1 mL MeCN:THF 80:20, add 70 μg panacyl bromide, add 1.025 μL N,N-diisopropylethylamine, mix, let stand at room temperature for 3 h, inject an aliquot onto column A (pre-equilibrated with 10 mL dichloromethane) and elute to waste with 15 mL dichloromethane, elute the contents of column A onto column B with the mobile phase and start the gradient, monitor the effluent from column B. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°).)

HPLC VARIABLES
Column: A Guard-Pak silica; B 250 × 4.6 5 μm Hibar Silica (Merck)
Mobile phase: Gradient. A was hexane:dichloromethane:THF:MeCN:MeOH 35:50:11:4:0.25. B was dichloromethane:MeOH 98:2. C was dichloromethane:MeOH:THF 92:7:1. A:B:C 100:0:0 for 35 min, to 0:100:0 over 10 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 15 min
Flow rate: 1
Injection volume: 20
Detector: F ex 253 em 445

CHROMATOGRAM
Retention time: 83
Limit of detection: 30 pg

OTHER SUBSTANCES
Simultaneous: alprostadil, 13,14-dihydro-15-ketoprostaglandin E_2, dinoprostone, 11-epi-prostaglandin E_2, 8-isoprostaglandin E_2, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin A_2, prostaglandin D_2, thromboxane B_2

KEY WORDS
derivatization; column-switching; normal phase

REFERENCE
Salari,H.; Yeung,M.; Douglas,S.; Morozowich,W. Detection of prostaglandins by high-performance liquid chromatography after conversion to *p*-(9-anthroyloxy)phenacyl esters, *Anal.Biochem.*, **1987**, *165*, 220–229.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Sep-Pak silica SPE cartridge with dichloromethane. Dissolve 0.04-100 ng compound and 50 ng IS in 1 mL MeCN:THF 80:20, add 10 μg panacyl bromide, add 1 μL triethylamine, mix, let stand at room temperature for 2 h, add to the SPE cartridge, wash with 20 mL dichloromethane, elute with 2 mL MeCN:MeOH 85:15. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: Dichloromethane:MeCN:MeOH 90:9:1
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 400 (cutoff filter)

CHROMATOGRAM
Retention time: 13.98
Internal standard: 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (7.14)
Limit of quantitation: 400 pg

OTHER SUBSTANCES
Simultaneous: dinoprostone, 6α-ketoprostaglandin $F_{1\alpha}$, thromboxane B_2

KEY WORDS
derivatization; SPE; normal phase

REFERENCE
Stein,T.A.; Angus,L.; Borrero,E.; Auguste,L.J.; Wise,L. High-performance liquid-chromatographic assay for prostaglandins with the use of *p*-(9-anthroyloxy)phenacyl bromide, *J.Chromatogr.*, **1987**, *395*, 591–595.

SAMPLE
Matrix: solutions
Sample preparation: Add 100 mg/mL 2-diethylaminoethyl chloride in MeCN:8% diethylisopropylamine in MeCN 1:10 to the sample, heat at 75° for 1 h .

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: Gradient. A was MeCN:water 30:70 containing 100 mM ammonium acetate. B was MeCN:water 70:30 containing 100 mM ammonium acetate. A:B from 100:0 to 0:100 over 20 min.
Flow rate: 1.2

Detector: UV 254, MS, Finnigan MAT 4500, Vestec thermospray interface, positive ion mode, vaporizer 224 ± 10°, source 250°, filament 1000 eV, emission current 150 μA, SIM, ion evaporation

CHROMATOGRAM
Retention time: 12.2
Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: dinoprostone, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin A_1, prostaglandin A_2, prostaglandin B_2, thromboxane D_2

KEY WORDS
derivatization

REFERENCE
Voyksner,R.D.; Bush,E.D.; Brent,D. Derivatization to improve thermospray HPLC/MS sensitivity for the determination of prostaglandins and thromboxane B2, *Biomed.Environ.Mass.Spectrom.*, **1987**, *14*, 523–531.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 Nucleosil 5 C18 + 250 × 4.6 μBondapak C18 in series
Mobile phase: MeCN:17 mM orthophosphoric acid 32.8:67.2
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Retention time: 35.5

OTHER SUBSTANCES
Simultaneous: dinoprostone, metabolites

REFERENCE
Hoult,J.R.S.; Bacon,K.B.; Osborne,D.J.; Robinson,C. Organ selective conversion of prostaglandin D_2 to 9α,11β-prostaglandin F_2 and its subsequent metabolism in rat, rabbit and guinea pig, *Biochem.Pharmacol.*, **1988**, *37*, 3591–3599.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Baker C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 20 μL of a solution in MeOH with 2.5 μL 20 mM 2,4-dimethoxyaniline hydrochloride in water and 5 μL 125 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH:pyridine 98.5:1.5, heat at 37° for 1 h, add 300 μL water, add to the SPE cartridge, wash with 2 mL buffer, wash with 1 mL MeOH:water 50:50, elute with 2 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot. (Buffer was MeOH:water 50:50 adjusted to pH 2.75 with HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:water 35:22:43 containing 500 μg/mL lithium chlorate, adjusted to pH 4.1 with trifluoroacetic acid
Flow rate: 1

Detector: E, Waters 460, thin-layer glassy-carbon working electrode 1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 14
Internal standard: 16,16-dimethylprostaglandin E_2 (30)
Limit of detection: 40-70 pg

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin D_2, thromboxane B_2

KEY WORDS
derivatization; SPE

REFERENCE
Knospe,J.; Steinhilber,D.; Herrmann,T.; Roth,H.J. Picomole determination of 2,4-dimethoxyanilides of prostaglandins by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1988**, *442*, 444–450.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL of a 0.01-10 μg/mL solution in MeOH with 100 μL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, let stand at room temperature for 1.5 h, inject a 5 μL aliquot. (Synthesis of 1-pyrenyldiazomethane is as follows. Suspend 5 g 1-pyrenecarboxaldehyde in 80 mL EtOH, add 3.4 g hydrazine monohydrate (Caution! Hydrazine monohydrate is a carcinogen!), stir at room temperature for 3 h, filter off the product and wash it with 50 mL cold EtOH, recrystallize from EtOH to obtain 1-pyrenecarboxaldehyde hydrazone as yellow crystals (mp 186-194° d). Add 6.55 g activated manganese dioxide to 2 g 1-pyrenecarboxaldehyde hydrazone in 300 mL diethyl ether, sonicate at room temperature for about 80 min (monitor by HPLC), filter, wash the solid with a little ether, evaporate the filtrate to obtain 1-pyrenyldiazomethane as red crystals. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971). 1-Pyrenyldiazomethane is also available from Molecular Probes, Eugene OR.)

HPLC VARIABLES
Column: 150 × 4 5 μm TSK-GEL-120A ODS (TOSOH)
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 5
Detector: F ex 340 em 395

CHROMATOGRAM
Retention time: 13
Limit of detection: 20-30 fmole

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization

REFERENCE
Nimura,N.; Kinoshita,T.; Yoshida,T.; Uetake,A.; Nakai,C. 1-Pyrenyldiazomethane as a fluorescent labeling reagent for liquid chromatographic determination of carboxylic acids, *Anal.Chem.*, **1988**, *60*, 2067–2070.

SAMPLE

Matrix: solutions

Sample preparation: Condition a Baker C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 100 μL of a solution in MeOH with 12.5 μL 20 mM 4-methoxyaniline hydrochloride in water and 25 μL 125 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH:pyridine 98.5:1.5, shake at 37° for 1 h, add 1.5 mL water (?), add to the SPE cartridge, wash with 2 mL buffer, wash with 1 mL MeOH:water 50:50, elute with 2 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH (J. Chromatogr. 1988, 442, 444), inject an aliquot. (Buffer was MeOH:water 50:50 adjusted to pH 2.75 with HCl.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:water 42:58, adjusted to pH 3.5 with trifluoroacetic acid

Flow rate: 1

Detector: UV 249

CHROMATOGRAM

Retention time: 14

Internal standard: 16,16-dimethylprostaglandin E_2 (35.5)

Limit of detection: 1.2-2.2 ng

OTHER SUBSTANCES

Simultaneous: dinoprostone, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, thromboxane B_2

KEY WORDS

derivatization; SPE

REFERENCE

Knospe,J.; Herrmann,T.; Steinhilber,D.; Roth,H.J. Derivatization of prostaglandins to corresponding anilides and analysis by HPLC, *Adv.Prostaglandin Thromboxane Leukot.Res.*, **1989**, *19*, 692–695.

SAMPLE

Matrix: solutions

Sample preparation: Mix sample with 400 μL 5 mM DBD-PZ in MeCN containing 70 mM diethylphosphorocyanidate, react for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene

(Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 45:55
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 12
Limit of detection: 12 fmol

OTHER SUBSTANCES

Simultaneous: alprostadil, hydrocortisone succinate
Interfering: prednisolone succinate

KEY WORDS

SPE; derivatization

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 100 μM solution of the carboxylic acid in water with 100 μL 100 mM 1-(3-methylaminopropyl)-3-ethylcarbodiimide in water, 100 μL 1% pyridine in water, and 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile

in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°) (Anal. Sci. 1989, 5, 525). 2-(5-Chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is also available from Dojindo, Kumamoto, Japan. Add 2 mL hydrazine hydrate to a stirred solution of 2 g 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in 20 mL anhydrous DMF (Caution! Hydrazine hydrate is a carcinogen!), stir at room temperature for 4 h, add 20 mL benzene (Caution! Benzene is a carcinogen!). Collect the precipitate and wash it with water and MeCN, recrystallize from DMF:benzene 50:50 to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as an off-white crystalline solid (mp >220° d).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil ODS-II, WS-II 5C18 HG
Mobile phase: MeCN:water 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 450

CHROMATOGRAM

Retention time: 65
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Simultaneous: alprostadil, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization

REFERENCE

Saito,M.; Chiyoda,Y.; Ushijima,T.; Sasamoto,K.; Ohkura,Y. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *Anal.Sci.*, **1994**, *10*, 679–681.

SAMPLE

Matrix: solutions
Sample preparation: 100 μL 10 mM compound in MeOH + 100 μL 1% pyridine in MeOH + 100 μL 15 mM reagent in DMSO, 100 μL 100 mM 1-ethyl-3-(3-dimethylaminopro-

pyl)carbodiimide in MeOH, heat at 37° for 1 h, inject a 10 μL aliquot. (Reagent was 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2-nitrophenyl)sulfide as yellow needles (mp 231-232°) (Anal. Sci. 1995, 11, 103). Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Reflux 5.0 g ethyl 2-chloromethyl-5-oxazolecarboxylate and 11.7 g NaI in 80 mL acetone for 1 h, partition the reaction mixture between ethyl acetate and water. Wash the organic layer with water and dry it over anhydrous sodium sulfate, evaporate to give ethyl 2-iodomethyl-5-oxazolecarboxylate as a reddish-brown oil. Reflux 7.4 g ethyl 2-iodomethyl-5-oxazolecarboxylate and 21.5 g silver carbonate in 100 mL THF:water 70:30 for 4 h, filter through Celite, evaporate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-hydroxymethyl-5-oxazolecarboxylate (mp 60.5-62°). Stir 2.04 g oxalyl chloride in 15 mL dichloromethane at -50° under nitrogen, add 1.54 g DMSO in 3 mL dichloromethane, after 5 min add 1.4 g ethyl 2-hydroxymethyl-5-oxazolecarboxylate in 6 mL dichloromethane, stir for 15 min at -50°, add 5.7 mL triethylamine, allow to warm to room temperature, dilute with dichloromethane, wash with water, dry over anhydrous sodium sulfate, concentrate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-carboxaldehyde-5-oxazolecarboxylate (mp 71.5-73°). Add 11.3 mL concentrated HCl to 750 mg di(4,5-dimethoxy-2-nitrophenyl)sulfide stirred in 100 mL EtOH, add 3.3 g tin powder at 40-45°, stir for 1 h at 40-45°, dilute with 100 mL water, pass hydrogen sulfide gas through this solution (Caution! Hydrogen sulfide is highly toxic!), filter, concentrate the filtrate under reduced pressure to give 4,5-dimethoxy-2-aminothiophenol. Take up this compound in 30 mL EtOH:acetic acid 2:1 and add 750 mg ethyl 2-carboxaldehyde-5-oxazolecarboxylate, reflux for 1 h, collect the precipitate and recrystallize it from EtOH to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as yellow needles (mp 200-201°). Add 381 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole to 20 mL EtOH containing 3 mL DMF and 5 mL hydrazine hydrate, reflux for 1 h, collect the precipitate and wash it with EtOH, dry under vacuum to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a yellow powder (mp 255.5-280° (d)).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 20 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 369 em 451

CHROMATOGRAM

Retention time: 30

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprostone, prostaglandin F1α

KEY WORDS
derivatization

REFERENCE
Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *J.Chromatogr.B*, **1995**, *674*, 167–175.

SAMPLE
Matrix: tissue
Sample preparation: Condition two Sep-Pak C18 SPE cartridges with water. Homogenize 1.5 g tissue with 22 mL 52.6 mM pH 7.52 Tris-HCl buffer. Mix 13 mL homogenate with 550 μL 70 μg/mL indomethacin in 1 mM sodium carbonate solution, vortex, shake at 120 rpm at 37° for 1 h, add 278 pmole dinoprost-d_4, add 50 μL 2 M HCl, add 430 μL EtOH, centrifuge at 4° at 1500 g for 10 min, add the supernatant to a SPE cartridge, wash with 5 mL MeCN:water 5:95, wash with 10 mL water, elute with 6 mL MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute with 200 μL pyridine, add 80 μL acetic anhydride, let stand at 5° under argon overnight, add 3.5 mL 10% acetic acid, add 500 μL MeCN, add the mixture to a SPE cartridge, wash with 3 mL MeCN:water 5:95, wash with 10 mL water, elute with 5 mL MeCN, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 100-5C18
Mobile phase: MeCN:100 mM formic acid:100 mM ammonium formate 60:8:32
Flow rate: 1
Injection volume: 20
Detector: MS, Vestec Model 750B HPLC-TSP-MS interface, positive ion mode, vaporizer control 146°, vaporizer tip 280°, vapour 323°, block 346°, tip heater 348°, m/z 301

CHROMATOGRAM
Retention time: 8
Internal standard: dinoprost-d_4 (8 min, m/z 305)
Limit of detection: 0.2 pmole

KEY WORDS
derivatization; rat; brain; SPE; method can be used for many related compounds (J.Chromatogr. 1991; 568; 11).

REFERENCE
Yamane,M.; Abe,A. High-performance liquid chromatography-thermospray mass spectrometry of hydroxy-polyunsaturated fatty acid acetyl derivatives, *J.Chromatogr.*, **1992**, *575*, 7–18.

SAMPLE
Matrix: tissue, urine
Sample preparation: Urine. Make up 24 h volume of urine to 20 mL with water, add 200 ng IS1, acidify to pH 4-5 with 2 M HCl, centrifuge, add 5 mL Amberlite XAD-2 (wetted form, wash with MeOH and water before use), stir at 0° for 30 min, place slurry in a sintered-glass funnel, wash with 20 mL water, force out residual water with nitrogen, elute with 10 mL MeOH. Concentrate the eluate to about 500 μL (mostly water) under a stream of nitrogen, extract twice with 1 mL ethyl acetate, evaporate to dryness, dissolve the residue in 30 μL diethyl ether:MeOH:acetic acid 90:10:0.5, add 200 μL diethyl ether:MeOH:acetic acid 100:1:0.5, add to SPE column, wash with 6 mL diethyl ether:MeOH:acetic acid 100:1:0.5, elute with 6 mL diethyl ether:MeOH:acetic acid 90:10:0.5. Evaporate

the eluate under a stream of nitrogen, dissolve the residue in 30 μL MeOH, inject a 5-10 μL aliquot. Tissue. Cut 100 mg tissue into small pieces, suspend in 2 mL 10 mM phosphate-buffered saline, heat at 37° for 30 min, add 2 mL 100 ng/mL IS1 (duodenum, kidney) or IS2 (stomach) in MeOH, adjust pH to 5 with 2 M acetic acid, extract with 5 mL chloroform. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in diethyl ether:acetic acid 100:0.5, add to SPE column, wash with 6 mL diethyl ether:acetic acid 100:0.5, elute with 6 mL diethyl ether:MeOH:acetic acid 90:10:0.5. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 30 μL MeOH, inject a 5-10 μL aliquot. (Prepare SPE column (1 mL bed volume) by adding a slurry of 70-230 mesh Kieselgel 60 (Merck) in diethyl ether:acetic acid 100:0.5 to a Pasteur pipette, wash with 6 mL diethyl ether:acetic acid 100:0.5.)

HPLC VARIABLES

Column: 250 × 4.6 Lichrospher 100 CH-18/2 RP-18
Mobile phase: MeCN:water:phosphoric acid 70:30:0.1
Flow rate: 1
Injection volume: 5-10
Detector: UV 193

CHROMATOGRAM

Retention time: 23
Internal standard: omega-nor-prostaglandin E2 (IS1, 16 min) or omega-homo-6-oxo-prostaglandin F1α (IS2)

OTHER SUBSTANCES

Extracted: dinoprostone, prostaglandins E3, D2, D3, thromboxane B2

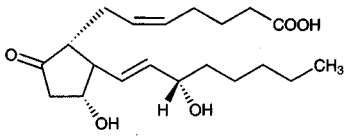
KEY WORDS

rat; SPE; duodenum; kidney; stomach

REFERENCE

Kivits,G.A.A.; Nugteren,D.H. The urinary excretion of prostaglandins E and their corresponding tetranor metabolites by rats fed a diet rich in eicosapentaenoate, *Biochim.Biophys.Acta*, **1988**, *958*, 289–299.

Dinoprostone



Molecular formula: $C_{20}H_{32}O_5$
Molecular weight: 352.47
CAS Registry No.: 363-24-6
Merck Index: 8064
Lednicer No.: 1 30

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Certify C18 SPE cartridge with water, MeCN, and 20 mM citric acid. Add 1 mL plasma to SPE cartridge, wash with 1 mL 20 mM citric acid, wash with 2 mL MeOH:water 10:90, wash with 2 mL cyclohexane, elute with 3 mL 3% ammonia in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 µL MeCN, add 200 µL 10 mM DBD-PZ in MeCN, add 300 µL 10 mM 2,2'-dipyridyl disulfide and 10 mM triphenylphosphine in MeCN, let stand at room temperature for 30 min, inject an aliquot. (DBD-PZ prepared from 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN added dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, extract three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ) as orange crystals, mp 121-2° (J. Chromatogr. 1991, 588, 61).)

HPLC VARIABLES

Column: 150 × 4.6 5 µm Inertsil ODS-2
Mobile phase: Gradient. MeCN:water from 35:65 to 60:40 over 1 h
Column temperature: 40
Flow rate: 1
Detector: F ex 440 em 569

CHROMATOGRAM

Retention time: 28.6
Limit of detection: 1.7-5 fmole

OTHER SUBSTANCES

Extracted: alprostadil (prostaglandin E1), dinoprost (prostaglandin F2α), limaprost, 6-ketoprostaglandin F1α, prostaglandin F1α, prostaglandin D2, prostaglandin A1, prostaglandin B1

KEY WORDS

plasma; rat; SPE; derivatization

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Terao,T.; Imai,K. Sensitive fluorometric detection of prostaglandins by high performance liquid chromatography after precolumn labelling with 4-(*N,N*-dimethylaminosulphonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ), *Biomed.Chromatogr.*, **1992**, *6*, 143–148.

SAMPLE

Matrix: blood

Sample preparation: 10 µL Serum + 44 µL MeOH + 1 µL pyridine, sonicate for 5 min, add 25 µL 100 mM reagent in DMF, add 20 µL 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, let stand at 25° for 2 h, centrifuge, inject an aliquot. (Reagent

was 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Pass dry hydrogen chloride into a mixture of 12.6 g methyl 2-furoate, 4.5 g paraformaldehyde, and 3.4 g anhydrous zinc chloride in 50 mL dry chloroform for 3 h while holding the reaction temperature at 30°. After cooling pour the contents of the flask into 100 mL cold water, remove the chloroform layer, extract the aqueous layer with chloroform (cf Coll. Czech. Chem. Commun. 1960, 25, 1058). Combine the chloroform layers, neutralize, dry over anhydrous calcium chloride, evaporate, distil to give 5-chloromethyl furyl-2-carboxylic acid methyl ester (bp 108°/4 mm Hg). Reflux 10 g 5-chloromethyl furyl-2-carboxylic acid methyl ester and 25 g silver carbonate in 100 mL THF: water 70:30 for 5 h, filter through Celite, concentrate the filtrate under reduced pressure, chromatograph the product on silica gel with chloroform to give 5-hydroxymethyl furyl-2-carboxylic acid methyl ester as a light yellow oil. Add a solution of 2.9 g 5-hydroxymethyl furyl-2-carboxylic acid methyl ester in 30 mL dichloromethane to 12 g pyridinium chlorochromate in 100 mL, dichloromethane, stir at room temperature for 4 h, evaporate to dryness under reduced pressure, chromatograph on silica with dichloromethane to give 5-formyl furyl-2-carboxylic acid methyl ester as a light yellow powder. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2- nitrophenyl)sulfide as yellow needles (mp 231-232°). Add 15 mL concentrated HCl dropwise to 1.5 g di(4,5-dimethoxy-2-nitrophenyl)sulfide and 4.5 g tin powder stirred at 40-50° in 150 mL EtOH, reflux for 1 h, cool to room temperature, filter, add 1.17 g 5-formyl furyl-2-carboxylic acid methyl ester to the filtrate, reflux for 1 h, cool, filter, chromatograph the solid on silica gel with dichloromethane, recrystallize from EtOH to give 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester as a yellow powder (mp 192-202°). Add 2 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 800 mg 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester in 20 mL EtOH, reflux for 30 min, collect the solid, wash with MeOH, dry under vacuum over phosphorus pentoxide to give 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a light yellow solid (mp 226-228°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 75:25 over 25 min, to 100:0 over 15 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 363 em 452

CHROMATOGRAM

Retention time: 50
Limit of detection: 50 fmole

OTHER SUBSTANCES

Extracted: alprostadil, arachidonic acid, dinoprost, lauric acid, linoleic acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, prostaglandin $F_{1\alpha}$, stearic acid

KEY WORDS

derivatization

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *Anal.Sci.*, **1995**, *11*, 103–107.

SAMPLE

Matrix: blood, tissue

Sample preparation: Purify blood or tissue homogenate using a Sep-Pak C18 SPE cartridge, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeCN:water:acetic acid 45:55:0.01
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Extracted: dinoprost, prostaglandin D2, 15-keto-prostaglandin E2, 13,14-dihydro-15-keto-prostaglandin F2α, 13,14-dihydro-15-keto-prostaglandin E2, 13,14-dihydro-15-keto-prostaglandin D2

KEY WORDS

rat; SPE

REFERENCE

Eguchi,N.; Kaneko,T.; Urade,Y.; Hayashi,H.; Hayaishi,O. Permeability of brain structures and other peripheral tissues to prostaglandins D_2, E_2 and $F_{2\alpha}$ in rats, *J.Pharmacol.Exp.Ther.*, **1992**, *262*, 1110–1120.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 0.4-3.1 mg dinoprostone in 1 mL MeCN, add 9.60 mg p-nitrophenacyl bromide, add 2.9 μL N,N-diisopropylethylamine, let stand at room temperature for 15 min, inject an aliquot.

HPLC VARIABLES

Column: two 250 × 2.1 Zorbax-Sil columns in series
Mobile phase: Dichloromethane:MeCN:DMF 80:20:0.5
Flow rate: 0.28
Detector: UV 254

CHROMATOGRAM

Retention time: 30.05

OTHER SUBSTANCES

Simultaneous: alprostadil

KEY WORDS

derivatization; normal phase

REFERENCE

Morozowich,W.; Douglas,S.L. Resolution of prostaglandin p-nitrophenacyl esters by liquid chromatography and conditions for rapid, quantitative p-nitrophenacylation, *Prostaglandins*, **1975**, *10*, 19–40.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 10 mg sample in 1 mL 15 mg/mL 2-bromo-4'-nitroacetophenone in MeCN, add 5 μL N,N-diisopropylethylamine, mix, let stand for 2 h at room temperature. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL chloroform, add 500 μL 200 mg/mL silver nitrate in water, mix thoroughly, centrifuge, filter (0.2 μm) the chloroform layer, inject a 4 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 1000 × 2.1 30-44 μm Vydac strong cation exchange resin containing silver (Prepare as follows. Equilibrate 6 g Vydac strong cation exchange resin with 600 mM silver nitrate in water, wash with water until no silver ion is detected, wash with 120 mL EtOH, wash with 120 mL acetone, wash with 120 mL ethyl acetate, wash with 120 mL 1,1,1-trichloroethane, wash with 120 mL hexane, dry under vacuum at 35-40° for 4 h, pack column.)

Mobile phase: Hexane:chloroform:MeCN 31.08:62.17:6.75

Column temperature: 26

Flow rate: 0.5

Injection volume: 4

Detector: UV 254

CHROMATOGRAM

Retention time: 10 (dinoprostone), 6 (trans-dinoprostone)

Limit of detection: 0.2%

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

derivatization

REFERENCE

Merritt,M.V.; Bronson,G.E. Determination of 5-trans-prostaglandin E2 in prostaglandin E2 via high performance liquid chromatography of their p-nitrophenacyl esters on a silver ion-loaded cation exchange resin, *Anal.Chem.*, **1976**, *48*, 1851–1853.

SAMPLE

Matrix: bulk

Sample preparation: Add 10 mg prostaglandin to 1 mL 15 mg/mL 2-bromo-4'-nitroacetophenone in MeCN, add 5 μL N,N-diisopropylethylamine, mix, let stand at room temperature for at least 2 h. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL chloroform, add 500 μL 200 mg/mL silver nitrate in water, mix thoroughly, centrifuge. Filter (0.2 μm) the chloroform layer and inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil SCX impregnated with silver ion (Prepare the column by pumping 80 mL 1 M silver nitrate in water through the column, wash the column with water until a negative test for silver ion is obtained, wash with 50 mL EtOH, wash with 50 mL acetone, wash with 50 mL ethyl acetate, wash with 50 mL trichloroethane, and wash with 50 mL hexane.)

Mobile phase: Dioxane:MeCN 99.94:0.06 (Caution! Dioxane is a carcinogen!)

Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.9

OTHER SUBSTANCES

Simultaneous: degradation products, alprostadil, dinoprost, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization

REFERENCE
Merritt,M.V.; Bronson,G.E. High-performance liquid chromatography of p-nitrophenacyl esters of selected prostaglandins on silver ion-loaded microparticulate cation-exchange resin, *Anal.Biochem.*, **1977**, *80*, 392–400.

SAMPLE
Matrix: bulk
Sample preparation: Mix 50-200 μg prostaglandins with 1 mL dry dichloromethane, 1 mg p-aminophenol, and 150 μL triethylamine, sonicate at 25° under nitrogen until dissolved, add at least a 3-fold molar excess of 2-bromo-1-methylpyridinium iodide, sonicate at 25° until the solution becomes cloudy, concentrate under a stream of nitrogen, add 1 mL 100 mM HCl, add 1 mL ethyl acetate, shake vigorously, centrifuge at 2500 rpm for 5 min, inject an aliquot of the supernatant. (Prepare 2-bromo-1-methylpyridinium iodide by analogy with the preparation of 2-chloro-1-methylpyridinium iodide. Add 15 g methyl iodide to 13.9 g 2-bromopyridine in 3 mL acetone at 0°, stir at room temperature for 3 days. Filter the precipitate and wash it with 50 mL dry ether, dry under reduced pressure to give 2-bromo-1-methylpyridinium iodide (Bull. Chem. Soc. Japan 1977, 50, 1863).)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Nucleosil C-18
Mobile phase: MeOH:water:perchloric acid 60:40:0.1 containing 50 mM sodium perchlorate
Column temperature: 25 ± 0.1
Flow rate: 1.1
Detector: E, 0.7 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: dinoprost

KEY WORDS
derivatization; guinea pig; plasma; human

REFERENCE
Ikenoya,S.; Hiroshima,O.; Ohmae,M.; Kawabe,K. Electrochemical detector for high performance liquid chromatography. IV. Analysis of fatty acids, bile acids and prostaglandins by derivatization to an electrochemically active form, *Chem.Pharm.Bull.(Tokyo)*, **1980**, *28*, 2941–2947.

SAMPLE
Matrix: bulk
Sample preparation: Make up a 20 μg/mL solution in dichloromethane, evaporate 1 mL under a stream of nitrogen, add 100 μL 20 mg/mL 2-bromo-2'-acetonaphthone in MeCN, add 100 μL 15 μL/mL N,N-diisopropylethylamine, add 100 μL MeCN, heat at 45° for 1 h, evaporate under a stream of nitrogen, take up the residue in 5 mL dichloromethane. (Purify α-bromo-2'-acetonaphthone in acetone solution with activated carbon, then recrystallize 2-bromo-2'-acetophenone from carbon tetrachloride.)

HPLC VARIABLES
Column: 250 × 4 Zorbax SIL
Mobile phase: Dichloromethane:MeCN:water:acetic acid:silver nitrate 87.5:12.5:0.4:0.2:0.17 (v:v:v:v:w)
Flow rate: 1
Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: 22

OTHER SUBSTANCES
Simultaneous: arbaprostil, meteneprost

KEY WORDS
derivatization; normal phase

REFERENCE
Kissinger,L.D.; Robins,R.H. Silver-modified mobile phase for normal-phase liquid chromatographic determination of prostaglandins and their 5,6-trans isomers in prostaglandin bulk drugs and triacetin solutions, *J.Chromatogr.*, **1985**, *321*, 353–362.

SAMPLE
Matrix: cell incubations
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. Centrifuge 4 mL of incubation at 1600 g for 5 min, add supernatant to the SPE cartridge, wash with 10 mL water, wash with 10 mL MeOH:water 15:85, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute in the minimum volume of EtOH:10 mM pH 7.4 phosphate buffer 50:50, inject an aliquot.

HPLC VARIABLES
Column: Nova Pak C18
Mobile phase: Gradient. A was MeOH:10 mM pH 7.4 phosphate buffer:t-amyl alcohol 6: 93.4:0.6. B was MeOH:t-amyl alcohol 99.4:0.6. A:B from 100:0 to 0:100 over 100 min
Flow rate: 1
Detector: radioactivity

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Extracted: arachidonic acid, leukotriene B4, 6-ketoprostaglandin F1α

KEY WORDS
radiolabeled; SPE

REFERENCE
Ohuchi,K.; Watanabe,M.; Fukui,Y.; Hirasawa,N.; Ozeki,T.; Tsurufuji,S. The effect of diphenylamine derivatives on arachidonic acid metabolism in rat peritoneal macrophages, *Prostaglandins Leukot.Med.*, **1987**, *28*, 15–23.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge 1 mL cell suspension at 1600 g for 10 min, add 3 mL EtOH to the supernatant, centrifuge at 0° at 2200 g for 10 min. Dilute the supernatant to an EtOH content of 15%, adjust to pH 3.0 with 1 M HCl, add to a Sep-Pak C18 SPE cartridge, wash with 20 mL water, wash with 20 mL EtOH:water 15:85, wash with 20 mL benzene (Caution! Benzene is a carcinogen!), elute with 20 mL ethyl acetate:MeOH 90:10, evaporate the eluate to dryness under reduced pressure at room temperature, reconstitute with 100 μL MeOH. Remove a 10 μL aliquot and add it to 10 μL 0.2% 9-anthryldiazomethane in ethyl acetate, let stand at room temperature overnight, add to a Sep-Pak silica SPE cartridge (conditioned with chloroform:toluene 50:50), elute with 10

mL MeCN:MeOH 80:20, evaporate to dryness under reduced pressure at room temperature, reconstitute in ethyl acetate:MeOH 50:50, inject an aliquot. (Synthesis of 9-anthryldiazomethane is as follows. Stir 8.8 g 9-anthraldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h, filter off the solid 9-anthraldehyde hydrazone and dry under vacuum (mp 124-6°) (Bull. Chem. Soc. Jpn. 1967, 40, 691). Dissolve 220 mg 9-anthraldehyde hydrazone in 100 mL anhydrous ether, add 800 mg activated manganese dioxide, follow the reaction by reverse-phase HPLC using MeCN at 0.4 mL/min and UV 254. At the end of the reaction filter off the manganese and wash it with 20 mL ether, evaporate the filtrate to obtain 9-anthryldiazomethane (mp 64-6°) (Anal.Biochem. 1980, 107, 116 and 1983, 132 456). Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971).)

HPLC VARIABLES

Guard column: 30 × 6 Develosil-ODS (Nomura Chemical)
Column: 250 × 4.6 Zorbax ODS
Mobile phase: MeCN:water:phosphoric acid 60:39.9:0.1
Column temperature: 30
Flow rate: 0.7
Detector: F ex 365 em 412

CHROMATOGRAM

Retention time: 30

OTHER SUBSTANCES

Extracted: 6-ketoprostaglandin E_2, 6-ketoprostaglandin $F_{1\alpha}$ prostaglandin D_2 , thromboxane B_2

KEY WORDS

derivatization; SPE

REFERENCE

Kiyomiya,K.; Yamaki,K.; Nimura,N.; Kinoshita,T.; Oh-Ishi,S. Phorbol myristate acetate-stimulated release of cyclooxygenase products in rat pleural cells: derivatization of prostaglandins with 9-anthryldiazomethane for fluorometric determination by high performance liquid chromatography, *Prostaglandins*, **1986**, *31*, 71–82.

SAMPLE

Matrix: cell suspensions

Sample preparation: Condition a 1 mL Bond Elut 5 μm ODS SPE cartridge with 2 mL diethyl ether, 2 mL MeOH, and 2 mL buffer. Condition a 1 mL 5 μm Bond Elut silica SPE cartridge with 2 mL MeOH:MeCN 15:85, with 2 mL acetone, and with 2 mL chloroform. Centrifuge at 4° at 12000 g for 10 min, acidify to pH 5.5 with 1% acetic acid, add to the ODS SPE cartridge, wash with 2 mL buffer, wash with 2 mL MeOH:buffer (pH 5.5), suck dry under vacuum for 2 min, wash with 2 mL petroleum ether, elute with 2 mL diethyl ether. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 20 μL MeOH, add 20 μL 1 mg/mL 9-anthroyldiazomethane in diethyl ether, heat at 37° for 6 h, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL chloroform, add to the silica SPE cartridge, wash with 3 mL chloroform, elute with 2 mL MeCN:MeOH 85:15. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, add 50 μL water, inject an aliquot. (Buffer was 0.0001% acetic acid adjusted to pH 5.5 with 1% NaOH. Synthesis of 9-anthryldiazomethane is as follows. Stir 10 g 9-anthraldehyde and 10 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 4 h, filter off the solid 9-anthraldehyde hydrazone and dry under vacuum. Dissolve 1 g 9-anthraldehyde hydrazone in 100 mL anhydrous ether, add 1 g activated manganese dioxide, add 500 μL saturated KOH in

EtOH, stir for 3 h, filter off the manganese, evaporate the filtrate to give 9-anthryldiazomethane. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971).))

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:water:acetic acid 62:37.9:0.1, pH adjusted to 5.5 with sodium acetate
Flow rate: 1
Detector: F ex 367 em 413

CHROMATOGRAM
Retention time: 23

OTHER SUBSTANCES
Extracted: dinoprost, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, thromboxane B_2

KEY WORDS
SPE; derivatization

REFERENCE
Wessel,K.; Kaever,V.; Resch,K. Measurement of prostaglandins from biological samples in the subnanogram range by fluorescence labelling and HPLC separation, *J.Liq.Chromatogr.*, **1988**, *11*, 1273–1292.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 500 μL enzyme incubation to 1 mL MeOH, mix, add 4 mL 100 mM citric acid, add 500 mg anhydrous sodium sulfate, extract twice (alprostadil, dinoprostone) or 3 times (dinoprost) with 5 mL portions of dichloromethane. Pass the extracts through 1 g anhydrous sodium sulfate and evaporate them to dryness, reconstitute with 1 mL anhydrous MeCN containing a 3-fold molar excess of α,p-dibromoacetophenone, add 2 μL diisopropylethylamine, let stand for 1 h, evaporate to dryness, reconstitute with 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Limit of quantitation: 5 μM

OTHER SUBSTANCES
Extracted: metabolites, alprostadil, dinoprost

KEY WORDS
derivatization

REFERENCE
Fitzpatrick,F.A. High-performance liquid chromatographic analysis of prostaglandins formed during in vitro incubations with prostaglandin 15-dehydrogenase, *J.Pharm.Sci.*, **1976**, *65*, 1609–1613.

SAMPLE

Matrix: enzyme incubations

Sample preparation: 2 mL Enzyme incubation + 2 mL MeOH, centrifuge. Remove the supernatant and add it to 2 mL 100 mM citric acid and 500 mg anhydrous sodium sulfate, extract twice with 5 mL portions of dichloromethane. Dry the extracts over anhydrous sodium sulfate and evaporate them to dryness under a stream of nitrogen, reconstitute with 100-200 μL 1.2 mg/mL p-bromophenacyl bromide in MeCN, add 0.5 μL diisopropylethylamine, let stand at room temperature for 1 h, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM

Retention time: 15
Limit of quantitation: <3 μg

OTHER SUBSTANCES

Extracted: dinoprost, prostaglandin A2, prostaglandin B2, prostaglandin D2, 15-methylprostaglandin B2

KEY WORDS

derivatization

REFERENCE

Fitzpatrick,F.A. High performance liquid chromatographic determination of prostaglandins F2α, E2, and D2 from in vitro enzyme incubations, *Anal.Chem.*, **1976**, *48*, 499–502.

SAMPLE

Matrix: enzyme incubations

Sample preparation: Condition a Sep-Pak silica SPE cartridge with dichloromethane. Centrifuge 1 mL enzyme incubation at 15000 g for 1 min, add 50 ng IS to the supernatant, add 1.5 mL cold (-20°) acetone, add 1.5 mL light petroleum, mix, discard the light petroleum layer, repeat the light petroleum wash. Adjust the pH of the aqueous phase to 3.5 with formic acid, extract twice with 1 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 45°, reconstitute the residue in 1 mL 10 μg/mL panacyl bromide in MeCN:THF 80:20, add 1 μL triethylamine, mix, let stand at room temperature for 2 h, add to the SPE cartridge, wash with 20 mL dichloromethane, elute with 2 mL MeCN:MeOH 85:15. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, inject a 20 μL aliquot. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid

with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°) (Anal. Biochem. 1987, 165, 220).)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: Dichloromethane:MeCN:MeOH 90:9:1
Flow rate: 2.3
Injection volume: 20
Detector: F ex 280 em 400 (cutoff filter)

CHROMATOGRAM
Retention time: 2.8
Internal standard: 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (2.1)
Limit of detection: 40 pg

KEY WORDS
derivatization; SPE; normal phase; rat; gastric mucosa

REFERENCE
Stein,T.A.; Angus,L.; Borrero,E.; Auguste,L.J.; Wise,L. Picogram measurement of prostaglandin E_2 synthesis by gastric mucosa by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *385*, 377–382.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with acetone, add hexanoic acid, heptanoic acid, and octanoic acid, add mixture to 5 g of a powdered 1:1 mixture of sodium sulfate and potassium bicarbonate + 2.7 mg dibenzo-18-crown-6 + 2.7 mg 4-bromomethyl-7-methoxycoumarin, let stand in the dark at 37° for 6 h, inject an aliquot.

HPLC VARIABLES
Column: 1060 × 0.2 3 μm Micro-Pak SP-18 (Varian)
Mobile phase: MeOH:MeCN:water 47.6:23.8:28.6
Column temperature: 35
Flow rate: 0.0006
Injection volume: 0.011
Detector: F ex 325 em 430 (laser-fluorescence)

CHROMATOGRAM
Retention time: 148
Internal standard: Hexanoic acid, heptanoic acid, octanoic acid
Limit of detection: 40-125 fmole

OTHER SUBSTANCES
Extracted: alprostadil (prostaglandin E1), prostaglandin D2, dinoprost (prostaglandin F2α), prostaglandin F1α, prostaglandin A2, prostaglandin B2, prostaglandin B1, prostaglandin A1

KEY WORDS
capillary; laser-fluorescence; derivatization

REFERENCE
McGuffin,V.L.; Zare,R.N. Femtomole analysis of prostaglandin pharmaceuticals, *Proc.Nat.Acad.Sci.USA*, **1985**, *82*, 8315–8319.

SAMPLE
Matrix: gastric mucosa

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL EtOH and 100 mL water. Homogenize gastric mucosa from three rats with 6 mL EtOH:50 mM pH 3.15 triethylammonium formate buffer 15:85 (cold), centrifuge at 300000 g for 15 min, resuspend the pellet in 3 mL EtOH:50 mM pH 3.15 triethylammonium formate buffer 15:85, centrifuge at 300000 g for 15 min. Combine the supernatants and add them to the SPE cartridge, wash with 50 mM pH 3.15 triethylammonium formate buffer 15:85, wash with 10 mL petroleum ether, elute with 10 mL methyl formate, store at -40°. Evaporate under helium, dissolve the residue in 100 μL diethyl ether with 2-3 drops MeOH, add 500 μL of a fresh solution of diazomethane, let stand in the dark at room temperature for 30 min, store in MeCN at -40°. (Prepare diazomethane from 1-methyl-3-nitro-1-nitrosoguanidine (Anal.Chem. 1973, 45, 2302).)

HPLC VARIABLES

Column: 150 × 4 5 μm Spherisorb ODS-2

Mobile phase: Gradient. A was 100 mM ammonium acetate adjusted to pH 3.5 with formic acid. B was MeCN:200 mM ammonium acetate buffer 2:1. A:B from 65:35 to 15:85 over 27 min.

Injection volume: 20

Detector: MS, Hewlett-Packard 5988A, thermospray, insertion probe 180-185°, SIM m/z 384

CHROMATOGRAM

Retention time: 26.7

Limit of detection: 110 pg

OTHER SUBSTANCES

Extracted: dinoprost, prostaglandin D2, 6-keto-prostaglandin F1α

KEY WORDS

rat; SPE; derivatization; LC-MS

REFERENCE

Abián,J.; Bulbena,O.; Gelpí,E. Thermospray liquid chromatography/mass spectrometry of prostaglandin methyl ester derivatives: application to the determination of prostaglandins E_2 and D_2 in rat gastric mucosa, *Biomed.Environ.Mass.Spectrom.*, **1988**, *16*, 215–219.

SAMPLE

Matrix: seminal fluid

Sample preparation: 1-5 μL Seminal fluid + 100 μL 5 μM IS in MeOH, mix, add 3 mL water to the supernatant, acidify to pH 3-4 with 100 mM HCl, extract with 7 mL ethyl acetate. Remove the ethyl acetate layer and evaporate it to dryness, reconstitute with MeOH. Evaporate to dryness in a clean tube, add 10 mg finely-powdered potassium bicarbonate:sodium sulfate 50:50, add 50 μL 0.4-1 mM 4-bromomethyl-7-acetoxycoumarin in acetone, add 50 μL 200 μM dibenzo-18-crown-6 in acetone, heat in the dark at 80° for 1 h, cool, inject a 20-40 μL aliquot. (Prepare 4-bromomethyl-7-acetoxycoumarin as follows. Reflux 50 g 7-hydroxy-4-methylcoumarin (β-methylumbelliferone) and 100 mL acetic anhydride for 1 h, cool, pour into 500 mL cold water, filter, dry the solid, recrystallize from EtOH to give 4-methyl-7-acetoxycoumarin. Reflux 10 g 4-methyl-7-acetoxycoumarin, 9 g N-bromosuccinimide, a little 2,2'-(azobis(2-methylpropionitrile) (α,α'-azobisisobutyronitrile, Eastman), and 100 mL carbon tetrachloride for 20 h, cool, evaporate under reduced pressure to remove the solvent, wash the residue with water, filter, dry, recrystallize from ethyl acetate/cyclohexane to give 4-bromomethyl-7-acetoxycoumarin (mp 184-185°) (J. Chromatogr. 1982, 234, 121).)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-18

Mobile phase: Gradient. MeCN:water from 30:70 to 90:10 over 99 min (Concave 1 curve (64 min) using a Japan Spectroscopic Model GP-A30 solvent programmer).

Column temperature: 50
Flow rate: 1
Injection volume: 20-40
Detector: F ex 365 em 460 following post-column reaction. The effluent from the column mixed with 100 mM NaOH pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 50° to the detector. (The prostaglandins are chromatographed as the coumarin derivatives then hydrolyzed in the post-column reactor to fluorescent 7-hydroxy-4-hydroxymethylcoumarin.)

CHROMATOGRAM
Retention time: 46
Internal standard: 16-methylprostaglandin $F_{1\alpha}$ (49)
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: dinoprost, alprostadil

KEY WORDS
derivatization; post-column reaction

REFERENCE
Tsuchiya,H.; Hayashi,T.; Naruse,H.; Takagi,N. Sensitive high-performance liquid chromatographic method for prostaglandins using a fluorescence reagent, 4-bromomethyl-7-acetoxycoumarin, *J.Chromatogr.*, **1982**, *231*, 247–254.

SAMPLE
Matrix: seminal fluid
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL EtOH and 20 mL water. Dilute seminal fluid to 1 mL, adjust to pH 3.5 with aqueous formic acid, centrifuge, add the supernatant to the SPE cartridge, wash with 20 mL EtOH:water 15:85, wash with 20 mL water, remove excess water mechanically, wash with 20 mL hexane, elute with 4 mL methyl formate. Dry the eluate under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot (J. Chromatogr. 1985, 349, 431). (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days (J. Chromatogr. 1983, 282, 435).)

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 C18
Mobile phase: Gradient. MeCN:0.5 mM formic acid 30:70 for 4.5 min, to 40:60 (step gradient).
Flow rate: 0.4
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Extracted: alprostadil, 19-hydroxyprostaglandin E_1, 19-hydroxyprostaglandin E_2, oxoprostaglandin E_1, oxoprostaglandin E_2

KEY WORDS
derivatization; SPE

REFERENCE

Doehl,J.; Greibrokk,T. Determination of prostaglandins in human seminal fluid by solid-phase extraction, pyridinium dichromate derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 21–32.

SAMPLE

Matrix: seminal fluid

Sample preparation: Mix 50 μL seminal fluid with 500 μL dilute HCl (pH 3.0) and 500 μL ethyl acetate, vortex. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL water, add to a Toyopak-ODS SPE cartridge, elute with 200 μL MeOH. 100 μL Eluate + 100 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water + 100 μL 1% aqueous pyridine + 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran is as follows. Slowly add 153 g freshly distilled phosphorus oxychloride to 73 g anhydrous DMF with stirring at room temperature, add 125 g sesamol in portions over 4 h, stir at room temperature overnight, pour into ice water, filter. Dissolve the solid in ether and wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as slightly-yellow crystals (mp 125-126°). Pass HCl gas into 15.6 g ethyl 2-thiophenecarboxylate, 4.5 g paraformaldehyde, and 3.4 g zinc chloride in 50 mL chloroform with stirring at 30° over 4 h. Pour into ice water and extract with 50 mL chloroform. Wash the chloroform layer 3 times with water, wash twice with aqueous sodium bicarbonate solution, dry over anhydrous sodium sulfate, evaporate to remove the solvent, distil at 86-94°/0.15 mm Hg to yield ethyl 5-chloromethyl thiophene-2-carboxylate as a colorless oil. Heat 3 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 3.68 g ethyl 5-chloromethyl thiophene-2-carboxylate, and 2.49 g potassium carbonate in 100 mL anhydrous DMF at 110° for 16 h, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with chloroform, recrystallize from chloroform:hexane 25:75 to give 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as yellow crystals (mp 124-126°). Heat 1.5 g 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 1.2 g hydrazine hydrate in 15 mL DMF at 70° for 1 h (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 10 g hydrazine hydrate, add 20 mL water, filter. Wash the solid with MeOH and dry it under reduced pressure to give 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as a yellow powder (mp 262-263°).)

HPLC VARIABLES

Column: 250 × 4 5 μm Wakosil ODS-II 5C18 HG
Mobile phase: MeCN:water 34:66
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 373 em 483

CHROMATOGRAM

Retention time: 123
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Extracted: alprostadil, dinoprost, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization; SPE

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Yakata,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran as novel fluorescence derivatization reagents for carboxylic acids in liquid chromatography, *Anal.Chim.Acta*, **1995**, *300*, 243–251.

SAMPLE

Matrix: seminal fluid

Sample preparation: Mix 50 μL seminal fluid with 500 μL dilute HCl (pH 3.0) and 500 μL ethyl acetate, vortex. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL water, add to a Toyopak-ODS SPE cartridge, elute with 200 μL MeOH. 100 μL Eluate + 100 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water + 100 μL 1% aqueous pyridine + 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran is as follows. Slowly add 153 g freshly distilled phosphorus oxychloride to 73 g anhydrous DMF with stirring at room temperature, add 125 g sesamol in portions over 4 h, stir at room temperature overnight, pour into ice water, filter. Dissolve the solid in ether and wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as slightly-yellow crystals (mp 125-126°). Pass HCl gas into 12.6 g methyl 2-furoate, 4.5 g paraformaldehyde, and 3.4 g zinc chloride in 50 mL chloroform with stirring at 30° over 4 h. Pour into ice water and extract with 50 mL chloroform. Wash the chloroform layer 3 times with water, wash twice with aqueous sodium bicarbonate solution, dry over anhydrous sodium sulfate, evaporate to remove the solvent, distil at 108°/4 mm Hg to yield methyl 5-chloromethyl furyl-2-carboxylate as a colorless oil. Heat 3 g 2-hydroxy-4,5- methylenedioxybenzaldehyde, 3.14 g methyl 5-chloromethyl furyl-2-carboxylate, and 2.49 g potassium carbonate in 100 mL anhydrous DMF at 110° for 16 h, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with chloroform, recrystallize from chloroform:hexane 25:75 to give 2-(5-methoxycarbonyl-2-furyl)-5,6-methylenedioxybenzofuran as slightly yellow crystals (mp 195-198°). Heat 1.42 g 2-(5-methoxycarbonyl-2-furyl)-5,6-methylenedioxybenzofuran and 1.2 g hydrazine hydrate in 15 mL DMF at 70° for 1 h (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 10 g hydrazine hydrate, add 20 mL water, filter. Wash the solid with MeOH and dry it under reduced pressure to give 2-(5-hydrazinocarbonyl-2-furyl)-5,6- methylenedioxybenzofuran as a light yellow powder (mp 261-262°).)

HPLC VARIABLES

Column: 250 × 4 5 μm Wakosil ODS-II 5C18 HG
Mobile phase: MeCN:water 34:66
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 362 em 462

CHROMATOGRAM

Retention time: 89
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Extracted: alprostadil, dinoprost, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization; SPE

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Yakata,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran as novel fluorescence derivatization reagents for carboxylic acids in liquid chromatography, *Anal.Chim.Acta*, **1995**, *300*, 243–251.

SAMPLE

Matrix: solutions

Sample preparation: Prepare methyl ester by treatment with excess ethereal diazomethane for 5 min, remove excess reagent under a stream of nitrogen. Dissolve 10 μg methyl ester in 200 μL anhydrous pyridine containing a 10-fold molar excess of p-nitrobenzylhydroxylamine hydrochloride, heat at 40° for 2 h, evaporate to dryness under a stream of nitrogen, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES

Column: 600 mm long μBondapak C18
Mobile phase: MeCN:water 85:15
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: alprostadil, prostaglandin A1, prostaglandin A2, prostaglandin B1, prostaglandin B2

KEY WORDS

derivatization

REFERENCE

Fitzpatrick,F.A.; Wynalda,M.A.; Kalser,D.G. Oximes for high-performance liquid and electron capture gas chromatography of prostaglandins and thromboxanes, *Anal.Chem.*, **1977**, *49*, 1032–1035.

SAMPLE

Matrix: solutions

Sample preparation: Condition a Sep-Pak silica SPE cartridge with 5 mL THF:water 95:5, 5 mL MeCN, and 5 mL dichloromethane. Prepare a solution in EtOH, add a 500 ng/mL solution of panacyl bromide in THF:MeCN 20:80, for each 1 mL of reaction mixture add 3 μL triethylamine, heat at 37° for 3 h, add 0.5 mL of the reaction mixture to the SPE cartridge, wash with 10 mL dichloromethane, elute with 3 mL MeCN:MeOH 85:15, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in MeCN, inject an aliquot. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid

with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°) (Anal.Biochem. 1987, 165, 220).)

HPLC VARIABLES
Column: 300 × 3.9 fatty acid analysis column (Waters?)
Mobile phase: Gradient. MeCN:water:acetic acid from 56:44:0.1 to 65:35:0.1 over 15 min
Flow rate: 1.2
Detector: F ex 249 em 413 (cut-off filter) or UV 254

CHROMATOGRAM
Retention time: 25.28
Limit of detection: 280 pg (UV), 50 pg (F)

OTHER SUBSTANCES
Simultaneous: dinoprost, prostaglandin D2, 6-keto-prostaglandin F1α, 6-keto-prostaglandin E1, 13,14-dihydro-15-keto-prostaglandin F2α

KEY WORDS
derivatization; SPE

REFERENCE
Watkins,W.D.; Peterson,M.B. Fluorescent/ultraviolet absorbing ester derivative formation and analysis of eicosanoids by high-pressure liquid chromatography, *Anal.Biochem.*, **1982**, *125*, 30–40.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10 equivalents of reagent, vortex for 1 min, let stand at room temperature for 8 min, add a volume of water equivalent to one tenth the volume of the reaction mixture, inject a 5 μL aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm pellicular C18 (Supelco)
Column: 200 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: Gradient. MeCN:10 mM formic acid from 40:60 to 60:40 over 10 min
Flow rate: 1.5
Injection volume: 5
Detector: UV 228 for 10 min then UV 298

CHROMATOGRAM
Retention time: 6
Limit of detection: 30-80 pmole

OTHER SUBSTANCES
Simultaneous: alprostadil, prostaglandin A_1, prostaglandin A_2, prostaglandin B_1, prostaglandin B_2

KEY WORDS
derivatization

REFERENCE
Dohl,J.; Greibrokk,T. High-performance liquid chromatographic separation and ultraviolet detection of prostaglandins, oxidized by pyridinium dichromate, *J.Chromatogr.*, **1983**, *282*, 435–442.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in 10 mM NaOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm PRP-1 styrene-divinylbenzene copolymer (Hamilton)
Mobile phase: MeCN:10 mM NaOH 21:79, measured pH 12.3 (At the end of each day wash the column with 40 mL water followed by 20 mL MeCN.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 206

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Simultaneous: dinoprost, prostaglandin A2, prostaglandin B2, prostaglandin D2, prostaglandin I2, thromboxane B2, 6-keto-prostaglandin F1α, 6-keto-prostaglandin E1

REFERENCE
Skrinska,V.; Thomas,G. High-performance liquid chromatography of prostacyclin, *J.Chromatogr.*, **1983**, *277*, 287–291.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in 10 mM NaOH, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm PRP-1 styrene-divinylbenzene copolymer (Hamilton)
Mobile phase: MeCN:10 mM NaOH 21:79, measured pH 12.3 (At the end of each day clean the column with 40 mL water and 20 mL MeCN.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 206

CHROMATOGRAM
Retention time: k' 3.0
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: 6-ketoprostaglandin F1α, 6-ketoprostaglandin E1, prostaglandin A2, prostaglandin B2, prostaglandin D2, epoprostenol, dinoprost, thromboxane B2

REFERENCE
Skrinska,V.; Thomas,G. High-performance liquid chromatography of prostacyclin, *J.Chromatogr.*, **1983**, *277*, 287–291.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, inject a 1 μL aliquot. Alternatively, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Column: 250 × 1.3 8 μm C18 (Chrompack)
Mobile phase: MeCN:10 mM pH 2.7 phosphoric acid 38:62
Flow rate: 0.06
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 25
Limit of detection: 0.14 pmole

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprost, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization; microbore

REFERENCE
Doehl,J.; Greibrokk,T. High-performance liquid chromatographic separation and determination of prostaglandins, oxidized by pyridinium dichromate. Optimization and applications, *J.Chromatogr.*, **1985**, *349*, 431–438.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of a solution in MeOH with 50 μL purified 9-anthryldiazomethane reagent, after 6 h inject an aliquot. (Purify 9-anthryldiazomethane on a 500 × 7.2 7 μm PG-pak C polystyrene gel column with ethyl acetate at 1 mL/min and UV 350 detection, inject 1 mg, collect the effluent when the purified compound elutes (20-22 min) and use it within 6 h.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm PG-Pak B silica gel
Mobile phase: Gradient. Isooctane:ethyl acetate:EtOH:acetic acid 90:10:0:1 for 15 min then 80:15:4:2 for 20 min (step gradient).
Flow rate: 1.2
Detector: F ex 365 em 412

CHROMATOGRAM
Retention time: 31.5
Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprost, HHT, hydroxyeicosatetraenoic acid, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, prostaglandin $F_{1\alpha}$, thromboxane B_2

KEY WORDS
derivatization; normal phase

REFERENCE
Yamauchi,Y.; Tomita,T.; Senda,M.; Hirai,A.; Terano,T.; Tamura,Y.; Yoshida,S. High-performance liquid chromatographic analysis of arachidonic acid metabolites by pre-column derivatization using 9-anthryldiazomethane, *J.Chromatogr.*, **1986**, *357*, 199–205.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.5 5 μm cyano (IBM)
Mobile phase: Gradient. Hexane:isopropanol 98:2 for 12 min, then to 80:20 over 10 min, maintain at 80:20
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM

Retention time: k' 8.59

OTHER SUBSTANCES

Extracted: arachidonic acid, prostaglandin H2, alprostadil (prostaglandin E1), prostaglandin D2, dinoprost (prostaglandin F2α), prostaglandin F1α, 6-ketoprostaglandin E1, 6-ketoprostaglandin F1α, thromboxane B2

REFERENCE

Zulak,I.M.; Puttemans,M.L.; Schilling,A.B.; Hall,E.R.; Venton,D.L. A fast, nondestructive purification scheme for prostaglandin H2 using a nonaqueous, bonded-phase high-performance liquid chromatography system, *Anal.Biochem.*, **1986**, *154*, 152–161.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve compound in 1 mL MeCN:THF 80:20, add 70 μg panacyl bromide, add 1.025 μL N,N-diisopropylethylamine, mix, let stand at room temperature for 3 h, inject an aliquot onto column A (pre-equilibrated with 10 mL dichloromethane) and elute to waste with 15 mL dichloromethane, elute the contents of column A onto column B with the mobile phase and start the gradient, monitor the effluent from column B. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80, add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°).)

HPLC VARIABLES

Column: A Guard-Pak silica; B 250 × 4.6 5 μm Hibar Silica (Merck)
Mobile phase: Gradient. A was hexane:dichloromethane:THF:MeCN:MeOH 35:50:11:4:0.25. B was dichloromethane:MeOH 98:2. C was dichloromethane:MeOH:THF 92:7:1. A:B:C 100:0:0 for 35 min, to 0:100:0 over 10 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 15 min
Flow rate: 1
Injection volume: 20
Detector: F ex 253 em 445

CHROMATOGRAM

Retention time: 63

Limit of detection: 30 pg

OTHER SUBSTANCES
Simultaneous: alprostadil, 13,14-dihydro-15-ketoprostaglandin E_2, dinoprost, 11-epiprostaglandin E_2, 8-isoprostaglandin E_2, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin A_2, prostaglandin D_2, thromboxane B_2

KEY WORDS
derivatization; column-switching; normal phase

REFERENCE
Salari,H.; Yeung,M.; Douglas,S.; Morozowich,W. Detection of prostaglandins by high-performance liquid chromatography after conversion to *p*-(9-anthroyloxy)phenacyl esters, *Anal.Biochem.*, **1987**, *165*, 220–229.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Sep-Pak silica SPE cartridge with dichloromethane. Dissolve 0.04-100 ng compound and 50 ng IS in 1 mL MeCN:THF 80:20, add 10 μg panacyl bromide, add 1 μL triethylamine, mix, let stand at room temperature for 2 h, add to the SPE cartridge, wash with 20 mL dichloromethane, elute with 2 mL MeCN:MeOH 85:15. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: Dichloromethane:MeCN:MeOH 90:9:1
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 400 (cutoff filter)

CHROMATOGRAM
Retention time: 7.90
Internal standard: 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (7.14)
Limit of quantitation: 40 pg

OTHER SUBSTANCES
Simultaneous: dinoprost, 6α-ketoprostaglandin $F_{1\alpha}$, thromboxane B_2

KEY WORDS
derivatization; SPE; normal phase

REFERENCE
Stein,T.A.; Angus,L.; Borrero,E.; Auguste,L.J.; Wise,L. High-performance liquid-chromatographic assay for prostaglandins with the use of *p*-(9-anthroyloxy)phenacyl bromide, *J.Chromatogr.*, **1987**, *395*, 591–595.

SAMPLE
Matrix: solutions
Sample preparation: Add 100 mg/mL 2-diethylaminoethyl chloride in MeCN:8% diethylisopropylamine in MeCN 1:10 to the sample, heat at 75° for 1 h .

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: Gradient. A was MeCN:water 30:70 containing 100 mM ammonium acetate. B was MeCN:water 70:30 containing 100 mM ammonium acetate. A:B from 100:0 to 0:100 over 20 min.

Flow rate: 1.2
Detector: UV 254, MS, Finnigan MAT 4500, Vestec thermospray interface, positive ion mode, vaporizer 224 ± 10°, source 250°, filament 1000 eV, emission current 150 μA, SIM, ion evaporation

CHROMATOGRAM
Retention time: 12.2
Limit of detection: 30 pg

OTHER SUBSTANCES
Simultaneous: dinoprost, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin A_1, prostaglandin A_2, prostaglandin B_2, thromboxane D_2

KEY WORDS
derivatization

REFERENCE
Voyksner,R.D.; Bush,E.D.; Brent,D. Derivatization to improve thermospray HPLC/MS sensitivity for the determination of prostaglandins and thromboxane B2, *Biomed.Environ.Mass.Spectrom.*, **1987**, *14*, 523–531.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 Nucleosil 5 C18 + 250 × 4.6 μBondapak C18 in series
Mobile phase: MeCN:17 mM orthophosphoric acid 32.8:67.2
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Retention time: 44

OTHER SUBSTANCES
Simultaneous: dinoprost, metabolites

REFERENCE
Hoult,J.R.S.; Bacon,K.B.; Osborne,D.J.; Robinson,C. Organ selective conversion of prostaglandin D_2 to 9α,11β-prostaglandin F_2 and its subsequent metabolism in rat, rabbit and guinea pig, *Biochem.Pharmacol.*, **1988**, *37*, 3591–3599.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Baker C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 20 μL of a solution in MeOH with 2.5 μL 20 mM 2,4-dimethoxyaniline hydrochloride in water and 5 μL 125 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH:pyridine 98.5:1.5, heat at 37° for 1 h, add 300 μL water, add to the SPE cartridge, wash with 2 mL buffer, wash with 1 mL MeOH:water 50:50, elute with 2 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot. (Buffer was MeOH:water 50:50 adjusted to pH 2.75 with HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:water 35:22:43 containing 500 μg/mL lithium chlorate, adjusted to pH 4.1 with trifluoroacetic acid

Flow rate: 1
Detector: E, Waters 460, thin-layer glassy-carbon working electrode 1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 15
Internal standard: 16,16-dimethylprostaglandin E_2 (30)
Limit of detection: 40-70 pg

OTHER SUBSTANCES
Simultaneous: dinoprost, prostaglandin D_2, thromboxane B_2

KEY WORDS
derivatization; SPE

REFERENCE
Knospe,J.; Steinhilber,D.; Herrmann,T.; Roth,H.J. Picomole determination of 2,4-dimethoxyanilides of prostaglandins by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1988**, *442*, 444–450.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 µL of a 0.01-10 µg/mL solution in MeOH with 100 µL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, let stand at room temperature for 1.5 h, inject a 5 µL aliquot. (Synthesis of 1-pyrenyldiazomethane is as follows. Suspend 5 g 1-pyrenecarboxaldehyde in 80 mL EtOH, add 3.4 g hydrazine monohydrate (Caution! Hydrazine monohydrate is a carcinogen!), stir at room temperature for 3 h, filter off the product and wash it with 50 mL cold EtOH, recrystallize from EtOH to obtain 1-pyrenecarboxaldehyde hydrazone as yellow crystals (mp 186-194° d). Add 6.55 g activated manganese dioxide to 2 g 1-pyrenecarboxaldehyde hydrazone in 300 mL diethyl ether, sonicate at room temperature for about 80 min (monitor by HPLC), filter, wash the solid with a little ether, evaporate the filtrate to obtain 1-pyrenyldiazomethane as red crystals. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971). 1-Pyrenyldiazomethane is also available from Molecular Probes, Eugene OR.)

HPLC VARIABLES
Column: 150 × 4 5 µm TSK-GEL-120A ODS (TOSOH)
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 5
Detector: F ex 340 em 395

CHROMATOGRAM
Retention time: 12
Limit of detection: 20-30 fmole

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprost, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization

REFERENCE
Nimura,N.; Kinoshita,T.; Yoshida,T.; Uetake,A.; Nakai,C. 1-Pyrenyldiazomethane as a fluorescent labeling reagent for liquid chromatographic determination of carboxylic acids, *Anal.Chem.*, **1988**, *60*, 2067–2070.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Baker C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 100 μL of a solution in MeOH with 12.5 μL 20 mM 4-methoxyaniline hydrochloride in water and 25 μL 125 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH:pyridine 98.5:1.5, shake at 37° for 1 h, add 1.5 mL water (?), add to the SPE cartridge, wash with 2 mL buffer, wash with 1 mL MeOH:water 50:50, elute with 2 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH (J. Chromatogr. 1988, 442, 444), inject an aliquot. (Buffer was MeOH:water 50:50 adjusted to pH 2.75 with HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:water 42:58, adjusted to pH 3.5 with trifluoroacetic acid
Flow rate: 1
Detector: UV 249

CHROMATOGRAM
Retention time: 17
Internal standard: 16,16-dimethylprostaglandin E_2 (35.5)
Limit of detection: 1.2-2.2 ng

OTHER SUBSTANCES
Simultaneous: dinoprost, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, thromboxane B_2

KEY WORDS
derivatization; SPE

REFERENCE
Knospe,J.; Herrmann,T.; Steinhilber,D.; Roth,H.J. Derivatization of prostaglandins to corresponding anilides and analysis by HPLC, *Adv.Prostaglandin Thromboxane Leukot.Res.*, **1989**, *19*, 692–695.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Baker C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 100 μL of a solution in MeOH with 12.5 μL 20 mM 4-methoxyaniline hydrochloride in water and 25 μL 125 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH:pyridine 98.5:1.5, shake at 37° for 1 h, add 1.5 mL water (?), add to the SPE cartridge, wash with 2 mL buffer, wash with 1 mL MeOH:water 50:50, elute with 2 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH (J. Chromatogr. 1988, 442, 444), inject an aliquot. (Buffer was MeOH:water 50:50 adjusted to pH 2.75 with HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:water 35:22:43 containing 500 μg/mL lithium chlorate, adjusted to pH 4.1 with trifluoroacetic acid
Flow rate: 1
Detector: E, Waters 460, glassy-carbon working electrode 1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 15

Internal standard: 16,16-dimethylprostaglandin E_2 (30)
Limit of detection: 45-75 pg

OTHER SUBSTANCES

Simultaneous: dinoprost, prostaglandin D_2, thromboxane B_2

KEY WORDS

derivatization; SPE

REFERENCE

Knospe,J.; Herrmann,T.; Steinhilber,D.; Roth,H.J. Derivatization of prostaglandins to corresponding anilides and analysis by HPLC, *Adv.Prostaglandin Thromboxane Leukot.Res.*, **1989**, *19*, 692–695.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a solution in DMF with 100 μL 62.5 mM N-hydroxysuccinimide in DMF and 100 μL 75 mM dicyclohexylcarbodiimide in DMF, let stand at 20° for 12 h, add 50 μL 2 mM coumarin 102 in DMSO, add 50 μL 0.5 mM luminarin 4 in DMSO, heat at 70° for 1 h, dilute 10-fold (or more) with DMSO, inject a 10 μL aliquot. (Luminarin 4, N-(4-aminobutyl)-2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetamide, may be obtained from Eurobio, Les Ulis, France. Synthesis is as follows. Reflux (with protection from moisture and with stirring) 2.12 g 8-hydroxyjulolidine, 2.22 g diethyl 1,3-acetonedicarboxylate (oxo-3-glutaric acid ethyl ester, Fluka), 1.71 g anhydrous zinc chloride, and 6 mL EtOH for 24 h, cool, add to 200 mL water, extract with 200 mL ethyl acetate, extract with 100 mL ethyl acetate. Combine the organic layers and wash them with water, dry over magnesium sulfate, evaporate to dryness, recrystallize from 5 parts ethyl acetate to give ethyl 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetate. Heat 2 g of this compound with 42 mL 1.2% NaOH in water and 40 mL MeOH at 45° for 1 h, cool, wash with 50 mL chloroform, wash with 40 mL chloroform. Degas the aqueous phase and acidify it with 16 mL 3 M HCl, stir for 15 min, adjust pH to 6.5 with 13 mL 2.5 M NaOH, filter. Wash the precipitate with water and dry it to obtain 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetic acid. Stir 11.26 g of this compound, 10.62 g disuccinimidyl oxalate (dihydroxysuccinimide carbonate), 3.81 g anhydrous triethylamine, and 560 mL dry MeCN protected from moisture at room temperature for 1 h, stir at 35-40° for 1 h, filter. Concentrate the filtrate and chromatograph on silica gel with dichloromethane:THF 50:50 to give luminarin 1 (21%). Stir 16.5 g luminarin 1 and 18.3 g 1,4-diaminobutane in 400 mL dry THF for 24 h, filter, evaporate the filtrate to dryness. Dissolve the residue in 100 mL dichloromethane and wash five times with 100 mL portions of water, evaporate to dryness, stir the residue with 25 mL dichloromethane to give luminarin 4 (US Pat. 5 151 517 (Sept. 9, 1992)).

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: MeCN:DMSO:5 (F) or 10 (chemiluminescence) mM pH 7 imidazole nitrate buffer 45:5:50 (F) or 45:0:55 (chemiluminescence)

Column temperature: 40 (chemiluminescence only)

Flow rate: 1.5 (F), 1.2 (chemiluminescence)

Injection volume: 10

Detector: F ex 390 em 470 (cut-off filter), Chemiluminescence. 1 mg/mL Bis(2,4,6-trichlorophenyl) oxalate in methyl acetate pumped at 0.25 mL/min and 400 mM hydrogen peroxide in THF pumped at 0.25 mL/min mixed in a 292 μL capillary tube at 40° and this mixture mixed with the column effluent. The resulting mixture flowed through a 60 μL PTFE capillary at 40° to a Kratos FS970 detector fitted with a 470 nm long-pass filter.

CHROMATOGRAM

Retention time: 4.5 (F), 10.5 (chemiluminescence)

Internal standard: coumarin 102 (Eastman) (9.0 (F), 15.0 (chemiluminescence))

Limit of detection: 300 fmole (F), 32 fmole (chemiluminescence)

OTHER SUBSTANCES
Simultaneous: isovaleric acid, nonanoic acid

KEY WORDS
derivatization

REFERENCE
Tod,M.; Prevot,M.; Chalom,J.; Farinotti,R.; Mahuzier,G. Luminarin 4 as a labelling reagent for carboxylic acids in liquid chromatography with peroxylate chemiluminescence detection, *J.Chromatogr.*, **1991**, *542*, 295–306.

SAMPLE
Matrix: solutions
Sample preparation: Mix 25 μL of an aqueous solution of fatty acids with 100 μL DMF: pyridine 93:7, add 50 μL 5 mM MPIB-hydrazide in DMF, add 100 μL 4 M (sic) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, heat at 40° for 20 min, inject a 10 μL aliquot. (Synthesis of MPIB-hydrazide, 4-(1-methylphenanthro[9,10-d]imidazol-2-yl)benzohydrazide, is as follows. Stir 1 g 9,10-diaminophenanthrene and 800 mg methyl 4-formylbenzoate (terephthaldehydic acid methyl ester) in 200 mL EtOH at room temperature for 1 h, add 5 mL MeOH saturated with HCl, reflux under an inert gas for 2 h, cool, concentrate to 50 mL under reduced pressure, chromatograph the precipitate on a 200 × 35 column of 70-230 mesh silica gel (ca. 100 g; Merck) with chloroform, recrystallize from MeOH to give methyl 4-(phenanthro[9,10-d]imidazol- 2-yl)benzoate as colorless needles (mp 312-315°). Dissolve 500 mg methyl 4-(phenanthro[9,10-d]imidazol-2-yl)benzoate in 100 mL anhydrous MeOH, treat with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 20 mL chloroform, chromatograph on a 200 × 60 column of about 250 g 100 mesh silica gel with chloroform to give methyl 4-(1-methylphenanthro[9,10-d]imidazol-2-yl)benzoate as colorless needles (mp 199-201°). Dissolve 2 g methyl 4-(1-methylphenanthro[9,10-d]imidazol-2-yl)benzoate in 100 mL aqueous hydrazine hydrate (45%) (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), heat at 100° for 1 h, recrystallize the precipitate from 95% EtOH to give MPIB-hydrazide (4-(1-methylphenanthro[9,10-d]imidazol-2- yl)benzohydrazide) (mp 291-293°).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSKgel ODS-80Ts (Tosoh)
Mobile phase: MeOH:water 80:20
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em 460, F ex 325 (10 mW He-Cd laser) em 460

CHROMATOGRAM
Retention time: 10.6
Limit of detection: 2.2-12.5 fmole (F), 0.4-2.3 fmole (laser F)

OTHER SUBSTANCES
Simultaneous: prostaglandin D_2, prostaglandin $E_{2\alpha}$, thromboxane A_2

KEY WORDS
derivatization

REFERENCE
Iwata,T.; Hirose,T.; Nakamura,M.; Yamaguchi,M. 4-(1-Methylphenanthro[9,10-*d*]imidazol-2-yl)benzohydrazide as derivatization reagent for carboxylic acids in high-performance liquid chromatography with conventional and laser-induced fluorescence detection, *Analyst*, **1994**, *119*, 1747–1751.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 100 μM solution of the carboxylic acid in water with 100 μL 100 mM 1-(3-methylaminopropyl)-3-ethylcarbodiimide in water, 100 μL 1% pyridine in water, and 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°) (Anal. Sci. 1989, 5, 525). 2-(5-Chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is also available from Dojindo, Kumamoto, Japan. Add 2 mL hydrazine hydrate to a stirred solution of 2 g 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in 20 mL anhydrous DMF (Caution! Hydrazine hydrate is a carcinogen!), stir at room temperature for 4 h, add 20 mL benzene (Caution! Benzene is a carcinogen!). Collect the precipitate and wash it with water and MeCN, recrystallize from DMF:benzene 50:50 to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as an off-white crystalline solid (mp >220° d).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil ODS-II, WS-II 5C18 HG
Mobile phase: MeCN:water 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 450

CHROMATOGRAM

Retention time: 78
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Simultaneous: alprostadil, dinoprost, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization

REFERENCE

Saito,M.; Chiyoda,Y.; Ushijima,T.; Sasamoto,K.; Ohkura,Y. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *Anal.Sci.*, **1994**, *10*, 679–681.

SAMPLE

Matrix: solutions

Sample preparation: 100 μL 10 mM compound in MeOH + 100 μL 1% pyridine in MeOH + 100 μL 15 mM reagent in DMSO, 100 μL 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, heat at 37° for 1 h, inject a 10 μL aliquot. (Reagent was 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2-nitrophenyl)sulfide as yellow needles (mp 231-232°) (Anal. Sci. 1995, 11, 103). Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Reflux 5.0 g ethyl 2-chloromethyl-5-oxazolecarboxylate and 11.7 g NaI in 80 mL acetone for 1 h, partition the reaction mixture between ethyl acetate and water. Wash the organic layer with water and dry it over anhydrous sodium sulfate, evaporate to give ethyl 2-iodomethyl-5-oxazolecarboxylate as a reddish-brown oil. Reflux 7.4 g ethyl 2-iodomethyl-5-oxazolecarboxylate and 21.5 g silver carbonate in 100 mL THF:water 70:30 for 4 h, filter through Celite, evaporate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-hydroxymethyl-5-oxazolecarboxylate (mp 60.5-62°). Stir 2.04 g oxalyl chloride in 15 mL dichloromethane at -50° under nitrogen, add 1.54 g DMSO in 3 mL dichloromethane, after 5 min add 1.4 g ethyl 2-hydroxymethyl-5-oxazolecarboxylate in 6 mL dichloromethane, stir for 15 min at -50°, add 5.7 mL triethylamine, allow to warm to room temperature, dilute with dichloromethane, wash with water, dry over anhydrous sodium sulfate, concentrate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-carboxaldehyde-5-oxazolecarboxylate (mp 71.5-73°). Add 11.3 mL concentrated HCl to 750 mg di(4,5-dimethoxy-2-nitrophenyl)sulfide stirred in 100 mL EtOH, add 3.3 g tin powder at 40-45°, stir for 1 h at 40-45°, dilute with 100 mL water, pass hydrogen sulfide gas through this solution (Caution! Hydrogen sulfide is highly toxic!), filter, concentrate the filtrate under reduced pressure to give 4,5-dimethoxy-2-aminothiophenol. Take up this compound in 30 mL EtOH:acetic acid 2:1 and add 750 mg ethyl 2-carboxaldehyde-5-oxazolecarboxylate, reflux for 1 h, collect the precipitate and recrystallize it from EtOH to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as yellow needles (mp 200-201°). Add 381 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole to 20 mL EtOH containing 3 mL DMF and 5 mL hydrazine hydrate, reflux for 1 h, collect the precipitate and wash it with EtOH, dry under vacuum to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a yellow powder (mp 255.5-280° (d)).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 20 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 369 em 451

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES
Simultaneous: alprostadil, dinoprost, prostaglandin F1α

KEY WORDS
derivatization

REFERENCE
Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *J.Chromatogr.B*, **1995**, *674*, 167–175.

SAMPLE
Matrix: tissue, urine
Sample preparation: Tissue. Homogenize 100 mg rat tissue with EtOH:100 mM HCl 80:20, centrifuge, wash the supernatant with petroleum ether, evaporate to dryness under reduced pressure, add 30 μg prednisolone, add 100 μL 2 mg/mL dansyl hydrazine in EtOH, add 100 μL EtOH:HCl 98:2, heat at 40° for 30 min, add 200 μL water, extract three times with 500 μL portions of ether. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 10 μL MeCN, inject a 0.1 μL aliquot. Urine. Activate silica gel (Wako gel C-100) at 120° for 4 h, cool. Suspend 5 g activated silica in toluene:ethyl acetate 90:10, pour into a 200 × 10 glass column. 50 mL Human urine + 30 μg prednisolone, mix, adjust pH to 3 with 1 M HCl, extract twice with 50 mL portions of ethyl acetate, Combine the organic layers and wash them with 100 mL 10 mM HCl, wash with 100 mL water. Evaporate the organic layer to dryness under reduced pressure, reconstitute with a small amount of toluene:ethyl acetate 90:10, add to the column, wash with 10 mL toluene:ethyl acetate 90:10, wash with 10 mL MeOH:ethyl acetate 5:100, elute with 20 mL MeOH:ethyl acetate 50:50, evaporate the eluate to dryness, add 100 μL 2 mg/mL dansyl hydrazine in EtOH, add 100 μL EtOH:HCl 98:2, heat at 40° for 30 min, add 200 μL water, extract three times with 500 μL portions of ether. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 10 μL MeCN, inject a 0.1 μL aliquot.

HPLC VARIABLES
Column: 160 × 1 10 μm μ Fine Pak SIL C18
Mobile phase: MeCN:MeOH:water:acetic acid 60:1:50:0.2
Flow rate: 0.008
Injection volume: 0.1
Detector: F ex 365 em 505

CHROMATOGRAM
Retention time: 11
Internal standard: prednisolone (14)
Limit of quantitation: <10 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; microbore; rat; brain; cerebrum; diencephalon; seminal vesicle; prostate; SPE

REFERENCE
Yamada,K.; Onodera,M.; Aizawa,Y. Determination of prostaglandin E_2 and the main prostaglandin E metabolite by micro high-performance liquid chromatography using fluorescence derivatization with dansyl hydrazine, *J.Pharmacol.Methods*, **1983**, *9*, 93–100.

SAMPLE
Matrix: tissue, urine

Sample preparation: Urine. Make up 24 h volume of urine to 20 mL with water, add 200 ng IS1, acidify to pH 4-5 with 2 M HCl, centrifuge, add 5 mL Amberlite XAD-2 (wetted form, wash with MeOH and water before use), stir at 0° for 30 min, place slurry in a sintered-glass funnel, wash with 20 mL water, force out residual water with nitrogen, elute with 10 mL MeOH. Concentrate the eluate to about 500 μL (mostly water) under a stream of nitrogen, extract twice with 1 mL ethyl acetate, evaporate to dryness, dissolve the residue in 30 μL diethyl ether:MeOH:acetic acid 90:10:0.5, add 200 μL diethyl ether:MeOH:acetic acid 100:1:0.5, add to SPE column, wash with 6 mL diethyl ether:MeOH:acetic acid 100:1:0.5, elute with 6 mL diethyl ether:MeOH:acetic acid 90:10:0.5. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 30 μL MeOH, inject a 5-10 μL aliquot. Tissue. Cut 100 mg tissue into small pieces, suspend in 2 mL 10 mM phosphate-buffered saline, heat at 37° for 30 min, add 2 mL 100 ng/mL IS1 (duodenum, kidney) or IS2 (stomach) in MeOH, adjust pH to 5 with 2 M acetic acid, extract with 5 mL chloroform. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in diethyl ether:acetic acid 100:0.5, add to SPE column, wash with 6 mL diethyl ether:acetic acid 100:0.5, elute with 6 mL diethyl ether:MeOH:acetic acid 90:10:0.5. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 30 μL MeOH, inject a 5-10 μL aliquot. (Prepare SPE column (1 mL bed volume) by adding a slurry of 70-230 mesh Kieselgel 60 (Merck) in diethyl ether:acetic acid 100:0.5 to a Pasteur pipette, wash with 6 mL diethyl ether:acetic acid 100:0.5.)

HPLC VARIABLES
Column: 250 × 4.6 Lichrospher 100 CH-18/2 RP-18
Mobile phase: MeCN:water:phosphoric acid 70:30:0.1
Flow rate: 1
Injection volume: 5-10
Detector: UV 193

CHROMATOGRAM
Retention time: 29
Internal standard: omega-nor-prostaglandin E2 (IS1, 16 min) or omega-homo-6-oxo-prostaglandin F1α (IS2)

OTHER SUBSTANCES
Extracted: dinoprost, prostaglandins E3, D2, D3, thromboxane B2

KEY WORDS
rat; SPE; duodenum; kidney; stomach

REFERENCE
Kivits,G.A.A.; Nugteren,D.H. The urinary excretion of prostaglandins E and their corresponding tetranor metabolites by rats fed a diet rich in eicosapentaenoate, *Biochim.Biophys.Acta*, **1988**, *958*, 289–299.

SAMPLE

Matrix: urine

Sample preparation: Urine. Make up 24 h volume of urine to 20 mL with water, add 200 ng IS, acidify to pH 4-5 with 2 M HCl, centrifuge, add 5 mL Amberlite XAD-2 (wetted form, wash with MeOH and water before use), stir at 0° for 30 min, place slurry in a sintered-glass funnel, wash with 20 mL water, force out residual water with nitrogen, elute with 10 mL MeOH. Concentrate the eluate to about 500 μL (mostly water) under a stream of nitrogen, extract twice with 1 mL ethyl acetate, evaporate to dryness, dissolve the residue in 30 μL diethyl ether:MeOH:acetic acid 90:10:0.5, add 200 μL diethyl ether:MeOH:acetic acid 100:1:0.5, add to SPE column, wash with 6 mL diethyl ether:MeOH:acetic acid 100:1:0.5, elute with 6 mL diethyl ether:MeOH:acetic acid 90:10:0.5. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 200 μL 0.5 M KOH, let stand at 20° for 1 h, adjust pH to 4 with 2 M acetic acid, extract with 2 mL diethyl ether, evaporate to dryness, add to another SPE column, wash with 6 mL hexane:diethyl ether:acetic acid 20:80:0.1, elute with 6 mL diethyl ether:MeOH:acetic acid 100:1:0.5, evaporate to dryness under a stream of nitrogen, reconstitute in 30 μL MeOH, inject a 5-10 μL aliquot. (Prepare SPE column (1 mL bed volume) by adding a slurry of 70-230 mesh Kieselgel 60 (Merck) in diethyl ether:acetic acid 100:0.5 to a Pasteur pipette, wash with 6 mL diethyl ether:acetic acid 100:0.5.)

HPLC VARIABLES

Column: 250 × 4.6 Lichrospher 100 CH-18/2 RP-18
Mobile phase: MeCN:water:acetic acid 62:38:0.1
Flow rate: 1
Injection volume: 5-10
Detector: UV 278

CHROMATOGRAM

Retention time: 38 (as prostaglandin B2)
Internal standard: omega-nor-prostaglandin E2 (21 min, as omega-nor-prostaglandin B2)

OTHER SUBSTANCES

Extracted: prostaglandins B1, B3

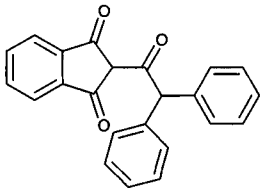
KEY WORDS

rat; SPE; derivatization

REFERENCE

Kivits,G.A.A.; Nugteren,D.H. The urinary excretion of prostaglandins E and their corresponding tetranor metabolites by rats fed a diet rich in eicosapentaenoate, *Biochim.Biophys.Acta*, **1988**, *958*, 289–299.

Diphenadione



Molecular formula: $C_{23}H_{16}O_3$
Molecular weight: 340.38
CAS Registry No.: 82-66-6
Merck Index: 3363

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 19.63

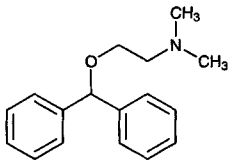
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Diphenhydramine



Molecular formula: $C_{17}H_{21}NO$
Molecular weight: 255.36
CAS Registry No.: 58-73-1, 147-24-0 (HCl), 88637-37-0 (citrate)
Merck Index: 3367
Lednicer No.: 1 41

SAMPLE

Matrix: blood
Sample preparation: Condition a 15 mg 3 mL PLUS.MPI (Ansys, USA) SPE disc with 200 μL MeOH and 200 μL 100 mM pH 6.0 potassium phosphate monobasic, do not allow to dry. Mix 1 mL serum with 1 mL 100 mM pH 6.0 potassium phosphate monobasic buffer, add to the SPE cartridge, wash with 500 μL 1 M acetic acid, wash with 500 μL MeOH, dry under vacuum for 5 min. Elute with two 300 μL portions of MeCN:triethylamine 100:2. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 800 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 1 opti-guard column RP C8
Column: 250 × 4.6 10 μm Chiralcel OD-R (Optimize Technologies, USA)
Mobile phase: MeCN:300 mM aqueous sodium perchlorate 42:58
Flow rate: 0.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 13.8

OTHER SUBSTANCES

Extracted: imipramine

KEY WORDS

diphenhydramine is IS; serum; SPE

REFERENCE

Liu,J.; Stewart,J.T. Quantitation of trimipramine enantiomers in human serum by enantioselective high-performance liquid chromatography and mixed-mode disc solid-phase extraction, *J.Chromatogr.B*, **1997**, *700*, 175–182.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 0.5 μg/mL imipramine hydrochloride + 200 μL saturated potassium carbonate, mix, add 5 mL hexane:isopropanol 98:2, shake at 230 rpm for 15 min, centrifuge at 800 g for 10 min. Remove the organic layer and add it to 100 μL 0.5% orthophosphoric acid, shake for 15 min, centrifuge at 800 g at 5° for 10 min, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeCN:water:1 M NaH_2PO_4 55:35:10
Flow rate: 1.8
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 4.6
Internal standard: imipramine (6.4)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma

REFERENCE
Selinger,K.; Prevost,J.; Hill,H.M. High-performance liquid chromatography method for the determination of diphenhydramine in human plasma, *J.Chromatogr.*, **1990**, *526*, 597–602.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Blood + 100 μL 1 μg/mL metycaine + 1 mL pH 9 saturated potassium borate buffer, mix, add 5 mL butyl chloride, extract. Remove the organic layer and add it to 1 mL 100 mM sulfuric acid, extract. Remove the aqueous layer and basify it with concentrated ammonium hydroxide, add 50 μL chloroform, extract. Remove the chloroform layer, evaporate to dryness under air at 60°, reconstitute in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Lichrospher RP-8
Mobile phase: MeCN:50 mM pH 3 phosphate buffer 45:55
Flow rate: 1.5
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6.3
Internal standard: metycaine (4)

OTHER SUBSTANCES
Simultaneous: sertraline, desmethylsertraline

REFERENCE
Logan,B.K.; Friel,P.N.; Case,G.A. Analysis of sertraline (Zoloft) and its major metabolite in postmortem specimens by gas and liquid chromatography, *J.Anal.Toxicol.*, **1994**, *18*, 139–142.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)

Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 2.8
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, encainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: doxepin, fentanyl, flecainide, nordoxepin, trazodone

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 259

CHROMATOGRAM

Retention time: 6.39
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 13.14
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 7.51
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluox-

etine, norpropoxyphene, northiaden, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: nortriptyline, sulforidazine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 1 mL Serum + 100 μL 1 mM orthophosphoric acid + 100 μL 1 M NaOH + 5 mL hexane, rotate at 60 rpm for 15 min, centrifuge. Remove 4 mL of the organic layer and add it to 80 μL 1 mM orthophosphoric acid, vortex vigorously for 30 s, inject a 50 μL aliquot of the aqueous phase. Urine. Dilute 1:10 with drug-free urine. 1 mL Diluted urine + 100 μL 1 mM orthophosphoric acid + 1 mL saturated sodium borate (pH 10.2) + 5 mL hexane, rotate at 60 rpm for 15 min, centrifuge. Remove 4 mL of the organic layer and add it to 80 μL 1 mM orthophosphoric acid, vortex vigorously for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelguard LC-CN cyanopropyl (Supelcosil)

Column: 150 × 4.6 5 μm Supelcosil LC-PCN cyanopropyl

Mobile phase: MeCN:MeOH:buffer 55:20:25 (Buffer was 2.6 g K_2HPO_4 in 1 L water, pH adjusted to 7.0 with 900 mM orthophosphoric acid.) (Optimize the separation by adjusting the pH of the mobile phase with a few drops of 1 M NaOH or 900 mM orthophosphoric acid.)

Flow rate: 2.5

Injection volume: 50

Detector: UV 205

CHROMATOGRAM

Retention time: 1.9

Internal standard: diphenhydramine

OTHER SUBSTANCES

Extracted: metabolites, nordiphenhydramine, meperidine, normeperidine

KEY WORDS

serum; diphenhydramine is IS

REFERENCE

Meatherall,R.C.; Guay,D.R.P.; Chalmers,J.L. Analysis of meperidine and normeperidine in serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 141–149.

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Grind 5 tablets to a fine powder, dissolve in 100 mL MeOH: 0.5% acetic acid 1:1, filter (paper), inject an aliquot. Suppositories. Cut up 3 suppositories, add to 100 mL MeOH:0.5% acetic acid 1:1, heat at 40° until all the fat melted, shake, filter (paper), inject a 25 μL aliquot. Liquid formulations. Dilute 10 mL formulation to 100 mL with MeOH:0.5% acetic acid 1:1, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: Gradient. A was 10 mM heptanesulfonic acid in 1 mM acetic acid. B was 10 mM heptanesulfonic acid and 1 mM acetic acid in MeOH. A:B from 60:40 to 25:75 over 30 min
Column temperature: 35
Flow rate: 1.75
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: dipyrone (metamizol), adiphenine, promazine, ethyldiphenacetate, drofenine, impurities

KEY WORDS
tablets; suppositories; liquid formulations

REFERENCE
Facchini,G.; Zaccheo,F.; Nannetti,M. Simultaneous determination of hydrochloride salts of adiphenine, diphenhydramine, ethyldiphenacetate, drofenine and promazine by ion-pair HPLC, *Boll.Chim.Farm.*, **1983**, *122*, 405–411.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water.

HPLC VARIABLES
Column: Bakerbond nitrile
Mobile phase: MeCN:water:triethylamine 50:50:0.5, adjusted to pH 6.5 with glacial acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.8

KEY WORDS
saline; injections; stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J.; Holland,J.S. Stability of dexamethasone sodium phosphate, diphenhydramine hydrochloride, lorazepam, and metoclopramide hydrochloride in portable infusion-pump reservoirs, *Am.J.Hosp.Pharm.*, **1994**, *51*, 514–517.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid
Column temperature: 30

Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 14.2

OTHER SUBSTANCES
Simultaneous: bromhexine, chlorpheniramine, codeine, dextromethorphan, ephedrine, papaverine, phenylephrine

KEY WORDS
syrup; indirect conductometric detection; presence of compound causes a decrease in mobile phase conductivity

REFERENCE
Lau,O.-W.; Mok,C.-S. High-performance liquid chromatographic determination of active ingredients in cough-cold syrups with indirect conductometric detection, *J.Chromatogr.A*, **1995**, *693*, 45–54.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 6.97

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300), mesna

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: ODS
Column: 250 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeCN:100 mM pH 4.5 KH_2PO_4 40:60
Flow rate: 1.2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 18.8
Limit of detection: 105 ng/mL

OTHER SUBSTANCES
Simultaneous: methyl paraben, ondansetron, propyl paraben

KEY WORDS
injections; saline

REFERENCE
Ye,L.; Stewart,J.T. HPLC determination of an ondansetron and diphenhydramine mixture in 0.9% sodium chloride injection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 711–718.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add N,N-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 μL aliquot of the supernatant. Urine. Inject a 100 μL aliquot of urine directly.

HPLC VARIABLES
Column: 150 × 4.6 Asahipak ODP-50 C18
Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 27:73
Flow rate: 0.8
Injection volume: 20-100
Detector: Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 13
Internal standard: N,N-dimethylbenzylamine
Limit of detection: 210 ng/mL

OTHER SUBSTANCES
Simultaneous: brompheniramine, chlorpheniramine, pyrilamine, pheniramine

KEY WORDS
tablets

REFERENCE
Holeman,J.A.; Danielson,N.D. Liquid chromatography of antihistamines using post-column tris(2, 2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, *679*, 277–284.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 100 × 8 4 μm Novapak C18
Mobile phase: MeCN:0.092%phosphoric acid + 0.2% triethylamine 26:74
Flow rate: 2
Detector: UV 214

CHROMATOGRAM
Internal standard: lidocaine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: diltiazem, metabolites
Also analyzed: bupivacaine

KEY WORDS
rat; liver

REFERENCE
Hussain,M.D.; Tam,Y.K.; Gray,M.R.; Coutts,K.T. Kinetic interactions of lidocaine, diphenhydramine, and verapamil with diltiazem: A study using isolated perfused rat liver, *Drug Metab.Dispos.*, **1994**, *22*, 530–536.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimet-

razine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: 8-chlorotheophylline, diphenylpyraline, promethazine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.2 5 μm Ultrasphere C18
Mobile phase: Gradient. A was MeCN containing 1 mg/mL heptanesulfonic acid. B was 50 mM pH 2.2 phosphoric acid containing 1 mg/mL heptanesulfonic acid. A:B 12.5:87.5 for 2.5 min, to 48.5:51.5 over 13.5 min, maintain at 48.5:51.5 for 4 min
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES
Simultaneous: dexamethasone, hydromorphone, creatinine, methyl paraben, propyl paraben, degradation products

KEY WORDS
stability-indicating; buffer

REFERENCE
Walker,S.E.; DeAngelis,C.; Iazzetta,J.; Eppel,J.G. Compatibility of dexamethasone sodium phosphate with hydromorphone hydrochloride or diphenhydramine hydrochloride, *Am.J.Hosp.Pharm.*, **1991**, *48*, 2161–2166.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil C8
Mobile phase: MeCN:water 55:45 containing 10 mM sodium dodecanesulfonate
Column temperature: 40
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: methyl salicylate

REFERENCE
Supelco Catalog, **1993,** p. 531.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: chlorcyclizine, chlorpheniramine, clonidine, promethazine, pyrilamine, triprolidine

REFERENCE
Supelco Catalog, **1994**, 768.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloro-

methiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Filter (1.2 μm) aliquots, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere-Cyano
Mobile phase: MeCN:MeOH:50 mM ammonium phosphate 55:10:35, pH adjusted to 3 with phosphoric acid
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 5

REFERENCE

Huang,H.-P.; Mehta,S.C.; Radebaugh,G.W.; Fawzi,M.B. Mechanism of drug release from an acrylic polymer-wax matrix tablet, *J.Pharm.Sci.*, **1994**, *83*, 795–797.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.16 μm PolyEncap ODS (n-octadecylacrylate copolymerized with vinyl silica in heptane, carrier Ultrasep ES 100; preparation described in paper)
Mobile phase: MeCN:pH 2.2 phosphate buffer 20:80
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: atropine, barbituric acid, codeine, noscapine, papaverine

REFERENCE

Engelhardt,H.; Cuñat-Walter,M.A. Polymer encapsulated stationary phases with improved efficiency, *Chromatographia*, **1995**, *40*, 657–661.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 10.14

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 12.20 (A), 6.01 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethyl-

perazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.72

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 3.4

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine

Interfering: methamphetamine, desipramine, nortriptyline

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B

Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 50:50 over 15 min.

Flow rate: 1.5

Injection volume: 20

Detector: UV 190-370

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

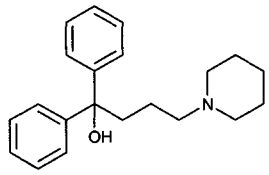
Extracted: morphine, ephedrine, phenylpropanolamine, cocaine, nortriptyline, lidocaine, benzoylecgonine, norpropoxyphene, nordiazepam

Also analyzed: amitriptyline, amphetamine, meperidine, codeine, (different gradient)

REFERENCE

Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

Diphenidol



Molecular formula: $C_{21}H_{27}NO$
Molecular weight: 309.45
CAS Registry No.: 972-02-1, 3254-89-5 (HCl), 26363-46-2 (pamoate)
Merck Index: 3369
Lednicer No.: 1 45

SAMPLE

Matrix: blood
Sample preparation: Add 600 μL 100 mM sodium carbonate to 100 μL plasma. Add 5 mL dichloromethane, shake on a reciprocal shaker for 10 min, centrifuge at 1000 g for 8 min, dry 4 mL of the lower organic phase in a rotary evaporator at 45°, dissolve the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-MS (Nacalai Tesque, Japan)
Mobile phase: MeOH:buffer 36:64 (Buffer was 0.3% triethylamine adjusted to pH 4.5 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 14.8
Internal standard: diphenidol

OTHER SUBSTANCES

Extracted: epinastine

KEY WORDS

plasma; diphenidol is IS

REFERENCE

Ohtani,H.; Kotaki,H.; Sawada,Y.; Iga,T. Quantitative determination of epinastine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 281–284.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 12.10 (A), 6.05 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

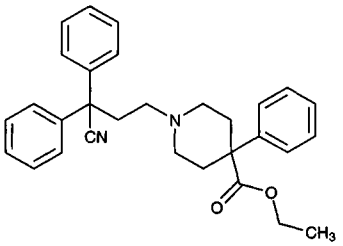
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Diphenoxylate



Molecular formula: $C_{30}H_{32}N_2O_2$
Molecular weight: 452.60
CAS Registry No.: 915-30-0, 3810-80-8 (HCl)
Merck Index: 3371
Lednicer No.: 1 302

SAMPLE

Matrix: formulations
Sample preparation: Oral solutions. Dilute an amount of oral solution equivalent to 2.5 mg diphenoxylate hydrochloride to 10 mL with EtOH. Tablets. Weight out finely powdered tablets equivalent to 2.5 mg diphenoxylate hydrochloride, add 10 mL MeCN:water 50:50, sonicate with frequent swirling for 15 min. Filter (0.45 μm), discard the first portion. Inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: A:B 34:66 (Prepare A as follows. Dissolve 192 mg 1-pentanesulfonic acid sodium salt monohydrate in 200 mL water. Add 800 mL water and 1 mL orthophosphoric acid. Prepare B as follows. Dissolve 192 mg 1-pentanesulfonic acid sodium salt monohydrate in 200 mL water. Add 800 mL MeCN and 1 mL orthophosphoric acid.)
Flow rate: 1.7
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: metabolites, atropine

KEY WORDS

oral solutions; tablets

REFERENCE

Lehr,G.J. Determination of diphenoxylate hydrochloride and atropine sulfate in combination drug formulations by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 1288–1293.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8

Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 14.10

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

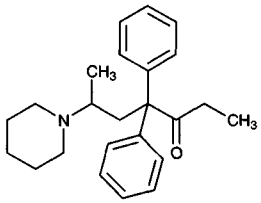
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Dipipanone



Molecular formula: $C_{24}H_{31}NO$
Molecular weight: 349.52
CAS Registry No.: 467-83-4, 856-87-1 (HCl)
Merck Index: 3398

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.8

OTHER SUBSTANCES

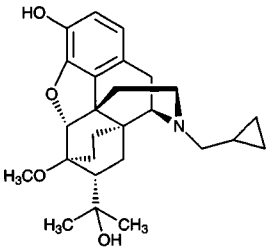
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ran-

itidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Diprenorphine



Molecular formula: $C_{26}H_{35}NO_4$
Molecular weight: 425.57
CAS Registry No.: 14357-78-9
Merck Index: 3403

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, pro-

mazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine,

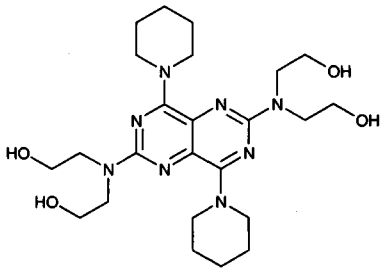
phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline,tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Dipyridamole

Molecular formula: $C_{24}H_{40}N_8O_4$
Molecular weight: 504.63
CAS Registry No.: 58-32-2
Merck Index: 3410
Lednicer No.: 1 428



SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 10.0 Tris buffer containing 150 ng/mL indomethacin + 8 mL diethyl ether, vortex 3 min, freeze for 1 h. Remove organic phase and evaporate it to dryness at room temperature. Dissolve residue in 200 µL MeOH, inject 20 µL aliquot.

HPLC VARIABLES

Column: 70 × 4.6 3 µm Ultrasphere XL ODS
Mobile phase: MeOH:20 mM ammonium acetate buffer (pH 5.0) 65:35
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 4.57
Internal standard: indomethacin (1.64)
Limit of detection: 10 ng/mL

KEY WORDS

plasma

REFERENCE

Barberi,M.; Merlin,J.L.; Weber,B. Sensitive determination of free and plasma protein-bound dipyridamole by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 511–515.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 285

CHROMATOGRAM

Retention time: 4.97

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 285.5

CHROMATOGRAM
Retention time: 13.197

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in MeOH to form an 0.1 to 0.3% solution

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:15 mM pH 7.6 NaH_2PO_4 from 60:40 to 90:10 over 15 min in a concave gradient (Perkin-Elmer Model 3B type 2)
Column temperature: 30
Flow rate: 2
Injection volume: 10
Detector: UV 280 or F ex 285 em 470

CHROMATOGRAM
Retention time: 3

REFERENCE
Fontani,F.; Finardi,G.P.; Targa,G.; Besana,G.P.; Ligorati,M. Purity evaluation of dipyridamole by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *280*, 181–187.

SAMPLE
Matrix: bulk
Sample preparation: 500 μL Plasma + 100 μL 5 mg/mL indomethacin in MeOH, filter (Sartorius SM 13243 ultrafiltration unit at 4000 g for 30 min). Add filtrate to a dry Chem Elut column (modified diatomaceous earth), leave 3 to 5 min, elute with 6 mL diethyl ether, evaporate eluant under a stream of nitrogen at room temperature, sonicate residue with 100 μL MeOH for 10 min, vortex, inject 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 55:45
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem 5100A dual electrode with an ESA guard cell, + 0.65 V

CHROMATOGRAM
Retention time: 9.62

Internal standard: indomethacin (11.74)
Limit of detection: 0.5 ng/mL

KEY WORDS
plasma

REFERENCE
Barberi-Heyob,M.; Merlin,J.L.; Pons,L.; Calco,M.; Weber,B. A sensitive isocratic liquid chromatography assay for the determination of dipyridamole in plasma with electrochemical detection, *J.Liq.Chromatogr.*, **1994**, *17*, 1837–1848.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injection with mobile phase to a final concentration 1 mg/mL. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetate buffer 65:35 (Buffer was 2.38 g sodium acetate in 350 mL water adjusted to pH 5.1 ± 0.1 with 36% acetic acid.)
Flow rate: 1
Injection volume: 15
Detector: UV 276

CHROMATOGRAM
Retention time: 23
Limit of quantitation: 500 μg/mL

KEY WORDS
injections; stability indicating

REFERENCE
Zhang,J.; Miller,R.B.; Russell,S.; Jacobus,R. Validation of a stability-indicating HPLC method for the determination of dipyridamole in dipyridamole injection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2109–2121.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to about 75 mg dipyridamole, add 40 mg pyrimethamine, dissolve in 20 mL MeCN, add 40 mL mobile phase, filter (paper), wash filter with mobile phase, make up filtrate to 100 mL with mobile phase. Dilute a 5 mL aliquot to 50 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:MeCN:water:triethylamine 55:5:40:0.1, pH adjusted to 4.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: pyrimethamine (3.5)
Limit of quantitation: 7.5 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin

KEY WORDS
tablets

REFERENCE
Sane,R.T.; Ghadge,J.K.; Jani,A.B.; Vaidya,A.J.; Kotwal,S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, *29*, 240–244.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ran-

itidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone,

phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 8.58 (A), 4.79 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, pro-

methazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

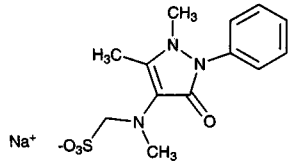
CHROMATOGRAM
Retention time: k' 2.51

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Dipyrone



Molecular formula: $C_{13}H_{16}N_3NaO_4S$
Molecular weight: 333.34
CAS Registry No.: 68-89-3, 5907-38-0 (monohydrate)
Merck Index: 3414
Lednicer No.: 2 262

SAMPLE

Matrix: blood
Sample preparation: 1-2 mL Plasma + 1 mL 40 μg/mL sodium salicylate in MeOH + 600-1000 mg potassium carbonate, shake for 1 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES

Column: 610 × 2 35-50 μm Bondapak AX/Corasil anion-exchange
Mobile phase: MeOH:pH 5.6 phosphate buffer 15:85
Flow rate: 0.5
Injection volume: 25-50
Detector: UV 254 for 9 min then UV 280

CHROMATOGRAM

Retention time: 6.3
Internal standard: sodium salicylate (11.2)
Limit of quantitation: 1000 ng/mL

KEY WORDS

plasma

REFERENCE

Asmardi,G.; Jamali,F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids, *J.Chromatogr.*, **1983**, *277*, 183–189.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL 15 mg/mL sodium bisulfite in water, let stand in an ice bath, add 1 mL 9 μg/mL hexobarbital in MeCN, mix for 10 s, centrifuge at 2000 g for 10 min. Evaporate 1 mL of the supernatant to dryness under reduced pressure (15 mmHg), reconstitute the residue in 70 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 23 × 3.8 30 μm Co-pell ODS
Column: 250 × 4 5 μm Fine SIL C-18 (Japan Spectroscopic Co.)
Mobile phase: MeCN:buffer 22:78 (Buffer was 10 mM KH_2PO_4 containing 1.24 mM tetra-n-butylammonium bromide, pH adjusted to 4.5 with 100 mM phosphoric acid.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 260

CHROMATOGRAM

Retention time: 8.2
Internal standard: hexobarbital (18.0)
Limit of detection: 700 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Itoh,S.; Tanabe,K.; Furuichi,Y.; Suzuka,T.; Kubo,K.; Yamazaki,M.; Kamada,A. Ion-pair high-performance liquid chromatographic analysis of sulpyrine and its metabolites in rabbit plasma, *Chem.Pharm.Bull.(Tokyo)*, **1984**, *32*, 3–3.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50-200 μL 50 μg/mL 4-propylaminoantipyrine in MeOH + 100 μL 1 M NaOH + 5 mL chloroform, vortex for 1 min, centrifuge at 850 g for 10 min, repeat the extraction. Evaporate the organic layers to dryness under a stream of air at 40°, reconstitute the residue in 50 μL MeOH, inject a 5-10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM sodium acetate 8:92, adjust to pH 3.0 with concentrated HCl
Flow rate: 1.6
Injection volume: 5-10
Detector: UV 257

CHROMATOGRAM
Retention time: 14 (as 4-methylaminoantipyrine, the hydrolysis product)
Internal standard: 4-propylaminoantipyrine (41)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, caffeine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Katz,E.Z.; Granit,L.; Drayer,D.E.; Levy,M. Simultaneous determination of dipyrone metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *305*, 477–484.

SAMPLE
Matrix: blood
Sample preparation: Serum + 330 mM perchloric acid, mix, centrifuge at 6600 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: MeCN:100 mM potassium phosphate adjusted to pH 3 with phosphoric acid 20:80
Flow rate: 1
Detector: UV 258

KEY WORDS
serum; pharmacokinetics

REFERENCE
Gascón,N.; Otal,C.; Martínez-Brú,C.; Mercé,J.; Cortés,M.; Arcelus,R.; Queraltó,J.M.; Sánchez,J.M.; González-Sastre,F. Dipyrone interference on several common biochemical tests, *Clin.Chem.*, **1993**, *39*, 1033–1036.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Plasma. 1-2 mL Plasma + 600-1000 mg potassium carbonate plasma + 1 mL 40 μg/mL sodium salicylate in MeOH, shake for 1 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 25-50 μL aliquot. Formulations. Grind tablets, weigh out amount equivalent to 5 mg dipyrone, dissolve in 100 mL water. Remove 3 mL of this solution and add it to 1 mL 40 μg/mL sodium salicylate in MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 610 × 2 25-50 μm Bondapak AX/Corasil anion exchange
Mobile phase: MeOH:pH 5.6 phosphate buffer 15:85
Flow rate: 0.5
Injection volume: 10-50
Detector: UV 254, 280

CHROMATOGRAM
Retention time: 6.3 (at UV 254)
Internal standard: sodium salicylate (11.2, at UV 280)
Limit of quantitation: 1000 ng/mL

KEY WORDS
plasma; tablets

REFERENCE
Asmardi,G.; Jamali,F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids, *J.Chromatogr.*, **1983**, *277*, 183–189.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out amount equivalent to 100 mg dipyrone, add 50 mL MeOH, sonicate for 2-3 min, make up to 100 mL with MeOH, filter (Whatman GF/C paper), dilute 1:100 with MeOH, mix an aliquot with an equal volume of 2 μg/mL phenacetin in MeOH, inject a 10 μL aliquot. Injections. Dilute with MeOH to a concentration of about 10 μg/mL, mix an aliquot with an equal volume of 2 μg/mL phenacetin in MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 50 mm long 10 μm RP-18
Column: 250 × 4.6 10 μm RP-18 (Merck)
Mobile phase: MeOH:water:triethylamine 50:50:0.025
Flow rate: 0.3
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 3.26
Internal standard: phenacetin (11.83)
Limit of detection: 4 ng

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets; injections; stability-indicating

REFERENCE
Eddine,N.H.; Bressolle,F.; Mandrou,B.; Fabre,H. Stability indicating assay for dipyrone. Part II. Separation and quantitative determination of dipyrone and its degradation products by high-performance liquid chromatography, *Analyst*, **1982**, *107*, 67–70.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind 5 tablets to a fine powder, dissolve in 100 mL MeOH:0.5% acetic acid 1:1, filter (paper), inject an aliquot. Suppositories. Cut up 3 suppositories, add to 100 mL MeOH:0.5% acetic acid 1:1, heat at 40° until all the fat melted, shake, filter (paper), inject a 25 μL aliquot. Liquid formulations. Dilute 10 mL formulation to 100 mL with MeOH:0.5% acetic acid 1:1, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: Gradient. A was 10 mM heptanesulfonic acid in 1 mM acetic acid. B was 10 mM heptanesulfonic acid and 1 mM acetic acid in MeOH. A:B from 60:40 to 25:75 over 30 min
Column temperature: 35
Flow rate: 1.75
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: diphenhydramine, adiphenine, promazine, ethyldiphenacetate, drofenine, impurities

KEY WORDS
tablets; suppositories; liquid formulations

REFERENCE
Facchini,G.; Zaccheo,F.; Nannetti,M. Simultaneous determination of hydrochloride salts of adiphenine, diphenhydramine, ethyldiphenacetate, drofenine and promazine by ion-pair HPLC, *Boll.Chim.Farm.*, **1983**, *122*, 405–411.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeCN:water 80:20, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3057 ODS silica (Hitachi)
Mobile phase: MeCN:water 25:75
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: caffeine, guaifenesin (guaiacol glycerol ether), acetaminophen, bucetin (3-hydroxy-p-butyrophenetidine), methyl p-hydroxybenzoate, phenacetin

KEY WORDS
semi-micro

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeOH, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3011 porous polymer (Hitachi)
Mobile phase: MeOH:ammonia 99:1
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.62

OTHER SUBSTANCES
Also analyzed: acetaminophen, caffeine, bucetin (3-hydroxy-p-butyrophenetidine), phenacetin, mefenamic acid, aspirin, salicylamide, salicylic acid, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS
semi-micro; porous polymer

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

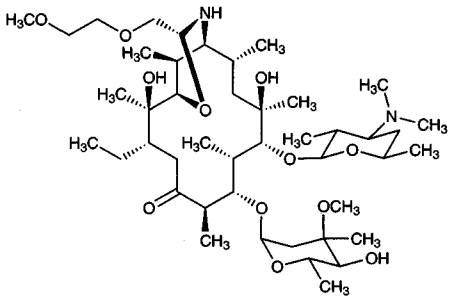
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dex-

tromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Dirithromycin



Molecular formula: $C_{42}H_{78}N_2O_{14}$
Molecular weight: 835.09
CAS Registry No.: 62013-04-1
Merck Index: 3418

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL pH 10 buffer (Fisher SO-B-116) + 10 μL 25 μg/mL IS in MeOH, mix for 10 s, add 5 mL dichloromethane, mix for 10-15 s, centrifuge at 2000 rpm for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN, inject a 40-60 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Sepralyte 2DP (Analytichem)
Mobile phase: MeCN:MeOH:water 60:10:30 containing 50 mM ammonium acetate, apparent pH 7.5
Column temperature: 40
Flow rate: 1
Injection volume: 40-60
Detector: E, ESA Coulochem Model 5100A, Model 5020 guard cell 1 V, Model 5011 analytical cell, first screening electrode 0.7 V, second electrode 0.9 V

CHROMATOGRAM

Retention time: 9
Internal standard: LY023907 (7)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: erythromycylamine

KEY WORDS

plasma; dog

REFERENCE

Whitaker,G.W.; Lindstrom,T.D. Determination of dirithromycin, LY281389 and other macrolide antibiotics by HPLC with electrochemical detection, *J.Liq.Chromatogr.*, **1988**, *11*, 3011–3020.

SAMPLE

Matrix: solutions
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil octadecylsilane
Mobile phase: MeCN:MeOH:50 mM pH 7.5 potassium phosphate buffer 44:19:37
Column temperature: 40
Flow rate: 2
Detector: RI

OTHER SUBSTANCES

Simultaneous: epidirithromycin

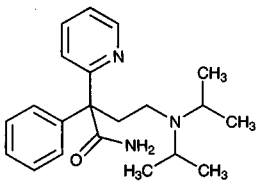
KEY WORDS

detector temp 40

REFERENCE

Kirst,H.A.; Creemer,L.C.; Paschal,J.W.; Preston,D.A.; Alborn,W.E.,Jr.; Counter,F.T.; Amos,J.G.; Clemens,R.L.; Sullivan,K.A.; Greene,J.M. Antimicrobial characterization and interrelationships of dirithromycin and epidirithromycin, *Antimicrob.Agents Chemother.*, **1995**, *39*, 1436–1441.

Disopyramide



Molecular formula: $C_{21}H_{29}N_3O$
Molecular weight: 339.48
CAS Registry No.: 3737-09-5, 22059-60-5 (phosphate)
Merck Index: 3424
Lednicer No.: 2 81

SAMPLE

Matrix: blood
Sample preparation: Alkalinize plasma with 1 M sodium hydroxide and extract with 1 mL chloroform (Caution! Chloroform is a carcinogen !). Centrifuge, remove organic layer and evaporate it to dryness under a stream of dry nitrogen. Reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 5 μm Brownlee CN
Mobile phase: MeCN:20 mM pH 4.0 monosodium phosphate 50:50
Flow rate: 1.0
Injection volume: 50
Detector: UV 207

CHROMATOGRAM

Internal standard: bidisomide
Limit of detection: 50 ng/mL

KEY WORDS

plasma; human; dog; pharmacokinetics

REFERENCE

Pao,L.-H.; Zhou,S.Y.; Cook,C.; Kararli,T.; Kirchhoff,C.; Truelove,J.; Karim,A.; Fleisher,D. Reduced systemic availability of an antiarrhythmic drug, bidisomide, with meal co-administration: Relationship with region-dependent intestinal absorption, *Pharm.Res.*, **1998**, *15*, 221–227.

SAMPLE

Matrix: blood
Sample preparation: To determine bound and unbound disopyramide mix 500 μL serum and 50 μL 30% trichloroacetic acid, centrifuge at 2000 g for 5 min. 100 μL supernatant + 25 μL 25 μg/mL benzocaine in water, vortex for 5 s, inject a 50 μL aliquot. To determine unbound disopyramide filter (Amicon Centrifree) while centrifuging at 2000 g for 10 min, 100 μL ultrafiltrate + 25 μL 25 μg/mL benzocaine in water, vortex for 5 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 μm μBondapak Radial Pak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 1.36 g sodium acetate trihydrate in 1 L water, pH adjusted to 4.5 with glacial acetic acid.)
Flow rate: 3
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 5
Internal standard: benzocaine (ethyl p-aminobenzoate) (9.5)
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Simultaneous: lidocaine, quinidine
Noninterfering: acetaminophen, amitriptyline, aspirin, chlorazepate, chlordiazepoxide, chlorpromazine, desipramine, diazepam, diphenhydramine, digoxin, doxepin, ephedrine, ethosuximide, flurazepam, glutethimide, imipramine, meperidine, meprobamate, methamphetamine, N-desisopropyl disopyramide, nordiazepam, nortriptyline, oxazepam, prazepam, procainamide, propoxyphene, propranolol, theophylline, trimipramine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Taylor,E.H.; Nelson,D.; Taylor,R.D.; Pappas,A.A. Rapid sample preparation and high performance liquid chromatographic determination of total and unbound serum disopyramide, *Ther.Drug Monit.*, **1986**, *8*, 219–222.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 16 μg/mL p-chlorodisopyramide in MeOH + 20 μL 1 M NaOH, vortex for 5 s, add 2 mL dichloromethane, shake for 5 min on a reciprocating shaker, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL 25 mM sulfuric acid, vortex for 5 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 2 silica gel 200 (Wako)
Column: 250 × 4 5 μm Develosil 60-5 (Nomura Chemical)
Mobile phase: MeCN:water 70:30 containing 15 mM $(NH_4)_2HPO_4$, pH 7.8
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 13
Internal standard: p-chlorodisopyramide (9)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetylprocainamide, atenolol, diltiazem, glycinexylidide, lidocaine, metoprolol, mexiletine, phenytoin, procainamide, propranolol, tocainide, verapamil

KEY WORDS
serum; pharmacokinetics

REFERENCE
Wang,L.H.; Kushida,K.; Ishizaki,T. Use of silica gel with aqueous eluent for simultaneous high performance liquid chromatographic assay of disopyramide and mono-N-dealkyldisopyramide, *Ther.Drug Monit.*, **1986**, *8*, 85–89.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min,

inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 261

CHROMATOGRAM

Retention time: 4.01
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. Mix 500 μL whole blood or plasma with 100 μL 1 M NaOH and 4 mL diethyl ether. Extract using a Labquake automatic shaker for 10 min, centrifuge at 1000 g for 5 min. Freeze the aqueous phase and remove the organic layer. Add 80 μL 100 mM phosphoric acid to the organic layer, vortex for 40 s, centrifuge, inject a 50 μL aliquot of the aqueous layer. Urine. Mix 500 μL urine with 2 mL 100 mM sodium carbonate and 4 mL diethyl ether. Extract using a Labquake automatic shaker for 10 min, centrifuge at 1000 g for 5 min. Freeze the aqueous phase and remove the organic layer. Add 80 μL 100 mM phosphoric acid to the organic layer, vortex for 40 s, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 6 μm Zorbax TMS

Mobile phase: MeCN:triethylamine:50 mM ammonium dihydrogen phosphate 15:0.5:85, adjusted to pH 2.6 with 1 M phosphoric acid

Flow rate: 1.1

Injection volume: 50

Detector: UV 262

CHROMATOGRAM

Retention time: 11

Internal standard: disopyramide

OTHER SUBSTANCES

Extracted: pirmenol

KEY WORDS

plasma; whole blood; disopyramide is IS

REFERENCE

Shand,D.G.; Verghese,C.; Barchowsky,A.; Hammill,S.C.; Pritchett,E.L.C. High-performance liquid chromatographic analysis of a new antiarrhythmic drug, pirmenol, in biological fluids, *J.Chromatogr.B*, **1981**, *224*, 343–347.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 60 μL 2 M NaOH + 6 mL water-saturated diethyl ether, extract for 15 min, centrifuge at 250 g for 3 min. Remove 5 mL of the ether layer and add it to 200 μL 1 mM phosphoric acid. Remove the aqueous phase and add it to 60 μL 100 mM NaOH, add 6 mL diethyl ether, extract for 15 min, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 110 μL mobile phase, inject a 50 μL aliquot. Urine. Sonicate the urine for 3 min. 500 μL Urine + 60 μL 5 M NaOH + 6 mL water-saturated diethyl ether, extract for 30 min, centrifuge at 250 g for 3 min. Remove 5 mL of the ether layer and add it to 200 μL 100 mM phosphoric acid. Remove the aqueous phase and add it to 60 μL 2 M NaOH, add 6 mL water-saturated diethyl ether, extract for 30 min, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 110 μL mobile phase, inject a 10-50 μL aliquot.

HPLC VARIABLES

Column: 30 × 3 Nucleosil C8 in series with 100 × 4 EnantioPac α1-acid glycoprotein (LKB)

Mobile phase: Isopropanol:10 mM pH 5.6 phosphate buffer 5:95

Column temperature: 23

Flow rate: 0.5

Injection volume: 10-50

Detector: UV 261

CHROMATOGRAM
Retention time: 13 (R), 18.5 (S)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral

REFERENCE
Enquist,M.; Hermansson,J. Comparison between two methods for the determination of the total and free (*R*)- and (*S*)-disopyramide in plasma using an α_1-acid glycoprotein column, *J.Chromatogr.*, **1989**, *494*, 143–156.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.445

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)

Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 100 mM KCl

Column temperature: 55

Flow rate: 0.6

Injection volume: 20
Detector: UV 263

OTHER SUBSTANCES

Also analyzed: lidocaine, metoprolol

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, pro-

mazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 amylose tris(3,5-dichlorophenylcarbamate)
Mobile phase: Hexane:EtOH 70:30
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: 17.5 (-), 20 (+)

KEY WORDS

chiral

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4 5 μm CHIRAL-AGP (ChromTech)
Mobile phase: Isopropanol:96 mM pH 4.1 acetate buffer 0.5:99.5
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Retention time: k' 2.78, 8.79 (enantiomers)

KEY WORDS

chiral

REFERENCE

Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column, *J.Chromatogr.A*, **1995**, *694*, 57–69.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.56 (A), 4.89 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

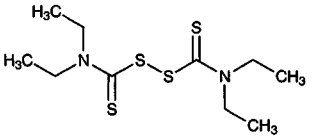
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Disulfiram



Molecular formula: $C_{10}H_{20}N_2S_4$
Molecular weight: 296.55
CAS Registry No.: 97-77-8
Merck Index: 3428
Lednicer No.: 1 223

SAMPLE

Matrix: blood

Sample preparation: For disulfiram. Inject a 100 μL aliquot of plasma directly onto column A with mobile phase A and elute to waste, after 4 min backflush the contents of column A onto column B with mobile phase B, after another 4 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent. For metabolites diethyldithiocarbamate and methyl diethyldithiocarbamate. 1 mL Plasma + 10 mM pH 7.5 phosphate buffer containing 200 mM EDTA + 1 μL 2-mercaptoethanol + 1 μL ethyl iodide, mix for 30 s, heat at 40° for 30 min, inject a 100 μL aliquot onto column A with mobile phase A and elute to waste, after 4 min backflush the contents of column A onto column B with mobile phase B, after another 4 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent. (Unchanged disulfiram was not found in plasma but the metabolites were found.)

HPLC VARIABLES

Column: A 50 × 3.9 40 μm Perisorb RP-18 (Merck); B 250 × 3.9 7 μm LiChrosorb RP-18
Mobile phase: A 10 mM pH 7.5 phosphate buffer containing 5 mM EDTA; B MeCN:10 mM pH 7.5 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 18.3 (disulfiram), 12.5 (diethyldithiocarbamate), 10.5 (methyl diethyldithiocarbamate)
Limit of detection: 3.7 ng/mL (diethyldithiocarbamate), 3.3 ng/mL (methyl diethyldithiocarbamate), 3 ng/mL (disulfiram)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; column-switching; derivatization

REFERENCE

Johansson,B. Rapid and sensitive on-line precolumn purification and high-performance liquid chromatographic assay for disulfiram and its metabolites, *J.Chromatogr.*, **1986**, *378*, 419–429.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 216.9

CHROMATOGRAM
Retention time: 25.13

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Inject a 2 μL aliquot of a solution in MeCN.

HPLC VARIABLES
Column: 150 × 1 3 μm 100 Å Reliasil (Michrome Bioresources)
Mobile phase: Gradient. MeCN:water:glycerol 50:48:2 for 15 min, to 80:18:2 over 10 min
Flow rate: 0.059
Injection volume: 2
Detector: UV 225 or MS, Finnigan MAT 95Q, CF-LSI-MS, cesium ions at 20 kV, 20:1 split; ion source 60

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: photolysis products

REFERENCE
Benson,L.M.; Veverka,K.A.; Mays,D.C.; Nelson,A.N.; Shriver,Z.H.; Lipsky,J.J.; Naylor,S. Simultaneous structure-activity determination of disulfiram photolysis products by on-line continuous-flow secondary ion mass spectrometry and enzyme inhibition assay, *J.Chromatogr.A*, **1995**, *693*, 162–166.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL

triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Adjust pH of 1-5 mL urine to 6.8 using 10 mM phosphate buffer, add 500 μL 100 mM lead (II) acetate solution, pump mixture onto column A at 1 mL/min and elute to waste, elute the contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent from column B. (Before the next injection flush column A with 5 mL MeCN then 5 mL of a buffer containing 10 mM potassium citrate and 10 mM EDTA).

HPLC VARIABLES

Column: A 4 × 2.1 5 μm Hypersil ODS; B 200 × 2.1 5 μm Hypersil ODS

Mobile phase: MeCN:10 mM pH 5.3 acetate buffer 65:35

Flow rate: A 1; B 0.5

Injection volume: 1000-5000

Detector: UV 435 following post-column derivatization. The column effluent flowed through a 2 × 4.6 reactor filled with metallic copper and a 4 × 4.6 reactor with filled copper(II) phosphate to the detector. (Metallic copper was prepared by adding 1 g Cu(I)Cl to 2 g sodium borohydride in 20 mL water, wash precipitate twice with water and MeOH, suspend in MeOH, sonicate, dry on tissue paper, pack firmly into column, use immediately (J.Chromatogr. 1986,370,439) (reactor lifetime 2 days). Copper(II) phosphate was prepared by mixing equal volumes of 100 mM potassium hydrogenphosphate (sic) and 100 mM copper(II) sulfate, wash precipitate twice with water and MeOH, suspend precipitate in MeOH, sonicate for 20 min, dry, pack in column (reactor lifetime 2 weeks).)

CHROMATOGRAM

Retention time: 5

Limit of detection: <1 ppm

OTHER SUBSTANCES

Extracted: metabolites, diethyldithiocarbamate (as copper complex)

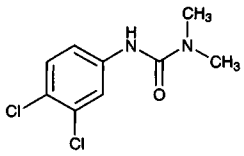
KEY WORDS

column-switching; post-column reaction

REFERENCE

Irth,H.; de Jong,G.J.; Brinkman,U.A.T.; Frei,R.W. Determination of disulfiram and two of its metabolites in urine by reversed-phase liquid chromatography and spectrophotometric detection after post-column complexation, *J.Chromatogr.*, **1988**, *424*, 95–102.

Diuron



Molecular formula: $C_9H_{10}Cl2N_2O$
Molecular weight: 233.10
CAS Registry No.: 330-54-1
Merck Index: 3447

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM

Retention time: 18.503

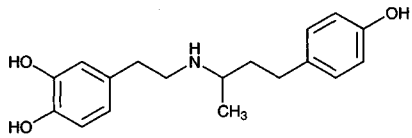
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Dobutamine



Molecular formula: $C_{18}H_{23}NO_3$

Molecular weight: 301.39

CAS Registry No.: 34368-04-2, 49745-95-1 (HCl), 104564-71-8 (lactobionate), 101626-66-8 (tartrate)

Merck Index: 3456

Lednicer No.: 2 53

SAMPLE

Matrix: blood

Sample preparation: Plasma. 1 mL Plasma + 125 μL 4 ng/mL isoproterenol + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 125 μL 40 ng/mL isoproterenol + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 20 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb ODS2

Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:20:60. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 52:48 for 6 min, go to 0:100 over 0.1 min, stay at 0:100 for another 10 min. Equilibrate at initial conditions for 4 min before next sample.

Flow rate: 1

Injection volume: 20-50

Detector: F ex 350 em 480

CHROMATOGRAM

Retention time: 16

Internal standard: isoproterenol (9)

Limit of detection: 200 pg/mL (urine), 8 pg/mL (plasma)

OTHER SUBSTANCES
Simultaneous: epinephrine, dopamine, epinine, norepinephrine, metabolites
Interfering: α-methyldopa

KEY WORDS
plasma

REFERENCE
Alberts,G.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of catecholamines and dobutamine in human plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *583*, 236–240.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma on ice + 200 μL 5% trichloroacetic acid, centrifuge at 1500 g for 10 min, filter (0.45 μm) supernatant, inject a 30 μL aliquot of the filtrate. (Plasma contained 1 mg/mL glutathione.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:buffer 20:80, pH adjusted to 2.7 with phosphoric acid (Buffer was 100 mM $NaH_2PO_4.H_2O$, 0.1 mM EDTA, 3 mM sodium octanesulfonate, and 1.5 mL/L triethylamine.)
Column temperature: 50
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem model 5100A, analytical cell ESA model 5011, electrode 1 350 mV, electrode 2 -260 mV.

CHROMATOGRAM
Retention time: 4
Limit of detection: 1 ng/mL

KEY WORDS
plasma

REFERENCE
Husseini,H.; Mitrovic,V.; Schlepper,M. Rapid and sensitive assay of dobutamine in plasma by high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.*, **1993**, *620*, 164–168.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: phenyl
Mobile phase: MeCN:20 mM phosphate buffer:glacial acetic acid 20:79.7:0.3
Flow rate: 2
Detector: UV 278

CHROMATOGRAM
Internal standard: methapyrilene

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE

Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.6 5 μm Lichrospher RP 18 end-capped
Mobile phase: MeCN:acetic acid:50 mM potassium phosphate buffer 20:10:70
Injection volume: 20
Detector: UV 280

OTHER SUBSTANCES

Simultaneous: dopamine

KEY WORDS

injections; 5% dextrose; stability-indicating

REFERENCE

Sautou-Miranda,V.; Gremeau,I.; Chamard,I.; Cassagnes,J.; Chopineau,J. Stability of dopamine hydrochloride and of dobutamine hydrochloride in plastic syringes and administration sets, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 186–193.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.58 (A), 3.80 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone,

methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

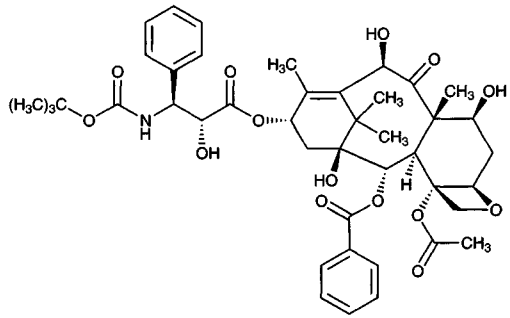
also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Docetaxel

Molecular formula: $C_{43}H_{53}NO_{14}$
Molecular weight: 807.89
CAS Registry No.: 114977-28-5
Merck Index: 3458



SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg IST endcapped cyano SPE cartridge (Sopachem BV, Netherlands) with 1 mL MeCN:triethylamine 100:0.1, 2 mL MeOH, and 2 mL 10 mM pH 5.0 ammonium acetate. Add 60 μL 10 μg/mL 2′-methylpaclitaxel in MeOH to 1200 μL plasma, mix for 20 s, centrifuge at 9500 g for 10 min, add 1 mL of the supernatant to the SPE cartridge at 0.4 mL/min. Wash with 2 mL 10 mM pH 5.0 ammonium acetate and 1 mL MeOH:water 20:80, elute with 500 μL MeCN:triethylamine 100:0.1, evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 100 μL MeCN:MeOH:water 40:10:50 by mixing for 30 s, inject a 50 μL aliguot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm APEX-Octyl (Jones Chromatography)
Mobile phase: MeCN:20 mM pH 5.0 ammonium acetate buffer 36.8:63.2
Flow rate: 1
Injection volume: 50
Detector: UV 227

CHROMATOGRAM

Retention time: 11.2
Internal standard: 2′-methylpaclitaxel (18.7)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, cimetidine, codeine, clemastine, diazepam, dexamethasone, diphenhydramine, flurazepam, oxazepam, paclitaxel, prednisolone, ranitidine

KEY WORDS

pharmacokinetics; plasma; SPE

REFERENCE

Rosing,H.; Lustig,V.; Koopman,F.P.; ten Bokkel Huinink,W.W.; Beijnen,J.H. Bio-analysis of docetaxel and hydroxylated metabolites in human plasma by high-performance liquid chromatography and automated solid-phase extraction, *J.Chromatogr.B*, **1997**, *696*, 89–98.

SAMPLE

Matrix: blood

Sample preparation: Add 5 mL MeCN:n-butyl chloride 20:80 to 1 mL plasma, vortex for 5 min, centrifuge at 4000 g for 5 min, evaporate the organic layer to dryness under a stream of nitrogen at 60°, reconstitute the residue with MeOH:water 50:50 with sonication for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A (GL Science, Japan)

Mobile phase: MeOH:THF:water:ammonium hydroxide 60:2.5:37.5:0.1, pH adjusted to 6.0 with formic acid
Column temperature: 60
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 8.5
Internal standard: docetaxel

OTHER SUBSTANCES
Simultaneous: paroxetine
Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, lorazepam, metoclopramide, morphine, ranitidine

KEY WORDS
pharmacokinetics; plasma; docetaxel is IS

REFERENCE
Sparreboom,A.; de Bruijn,P.; Nooter,K.; Loos,W.J.; Stoter,G.; Verweij,J. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *705*, 159–164.

SAMPLE
Matrix: formulations
Sample preparation: Add docetaxel injection to 0.9% NaCl injection or 5% dextrose injection to make a docetaxel concentration of 560 or 960 mg/mL, mix thoroughly. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere RP18
Mobile phase: MeOH:water 90:10
Column temperature: 28
Flow rate: 1.3
Injection volume: 500
Detector: UV 273

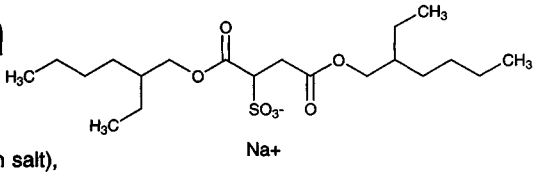
CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Simultaneous: polysorbate 80, diethylhexyl phthalate

KEY WORDS
injections

REFERENCE
Mazzo,D.J.; Nguyen-Huu,J.-J.; Pagniez,S.; Denis,P. Compatibility of docetaxel and paclitaxel in intravenous solutions with polyvinyl chloride infusion materials, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 566–569.

Docusate sodium



Molecular formula: $C_{20}H_{37}NaO_7S$
Molecular weight: 444.57
CAS Registry No.: 577-11-7, 7491-09-0 (potassium salt), 128-49-4 (calcium salt)
Merck Index: 3460

SAMPLE

Matrix: formulations
Sample preparation: Place a 100 mg capsule in 1 mL water, heat to dissolve, add 2 mL 2 mg/mL progesterone in MeOH, make up to 100 mL with MeOH, allow to settle, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 4.6 10 μm 100 Å Chromegabond C-22 (ES Industries)
Mobile phase: MeCN:water 70:30 containing 5 mM tetrabutylammonium phosphate (Pic A)
Flow rate: 1.8
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Retention time: 4.5
Internal standard: progesterone (6.8)

OTHER SUBSTANCES

Noninterfering: ferrous fumarate, casanthranol

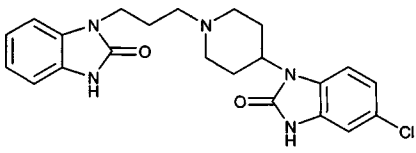
KEY WORDS

capsules

REFERENCE

Hogue,D.R.; Zimmardi,J.A.; Shah,K.A. High-performance liquid chromatographic analysis of docusate sodium in soft gelatin capsules, *J.Pharm.Sci.*, **1992**, *81*, 359–361.

Domperidone



Molecular formula: $C_{22}H_{24}ClN_5O_2$
Molecular weight: 425.92
CAS Registry No.: 57808-66-9
Merck Index: 3476
Lednicer No.: 3 174

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 12.355

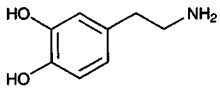
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Dopamine



Molecular formula: $C_8H_{11}NO_2$
Molecular weight: 153.18
CAS Registry No.: 51-61-6, 62-31-7 (HCl)
Merck Index: 3479

SAMPLE

Matrix: blood
Sample preparation: Mix 450 μL plasma with 50 μL 310 μ g/L iso-homovanilic acid in antioxidant solution. Dialyze this solution using a Carnegie Medicin (Stockholm) microdialysis system. Perfuse 10 mm microdialysis probes with Ringer solution at 2 μ L/min. Collect dialysate for each plasma sample over 20 min in a vial containing 80 μL antioxidant solution, inject a 100 μL aliquot. (Antioxidant solution was 10 mM HCl containing 1 g/L sodium metabisulfite and 0.1 g/L Na2EDTA.)

HPLC VARIABLES

Guard column: 30 × 4 Bondapak C18/Corasil 37-50 μ m
Column: 250 × 4.8 5 μm ODS(Beckman,CA)
Mobile phase: MeOH:buffer 20:80 (Buffer was 70 mM pH 2.55 NaH_2PO_4 containing 2.08 mM sodium octanesulfonate and 80 μ M EDTA.)
Flow rate: 1
Injection volume: 100
Detector: E , Waters 460 containing an electrochemical cell fitted with a glassy carbon working electrode and an Ag/AgCl reference electrode; the detector potential is + 0.8 V°vs the reference electrode

CHROMATOGRAM

Retention time: 5.5
Internal standard: iso-homovanilic acid (10)
Limit of detection: 0.4 nM/L

OTHER SUBSTANCES

Extracted: dihydroxyphenylacetic acid, homovanilic acid, levodopa

KEY WORDS

plasma; pharmacokinetics; dialysate

REFERENCE

Dethy,S.; Laute,M.A.; Van Blercom,N.; Damhaut,P.; Goldman,S.; Hildebrand,J. Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients, *Clin.Chem.*, **1997**, *43*, 740–744.

SAMPLE

Matrix: blood
Sample preparation: 20 mL Whole blood + 1 mL 20 mg/mL EDTA solution containing 10 mg/mL sodium metabisulfite, mix, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and add concentrated perchloric acid until the concentration of perchloric acid is 400 mM, mix, let stand in the cold for 15 min, centrifuge at 4° at 20000 g for 20 min. Adjust pH of 2 mL supernatant to 7.0 ± 0.2 with 500 mM KOH, add 400 μL 875 μg/mL o-phthalaldehyde in pH 10.40 ± 0.02 buffer (containing 2-mercaptoethanol ?), add 2 g NaCl, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers and add them to 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, discard the aqueous layer, wash the ethyl acetate layer again with phosphate buffer. Reduce the ethyl acetate volume to 100 μL under a stream of nitrogen, inject a 10-50 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 buffer 25:75 for 15 min then MeOH:25 mM pH 5.10 NaH_2PO_4 buffer 45:55 (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 29
Internal standard: tyramine (44)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: norepinephrine, serotonin

KEY WORDS
plasma; whole blood; pig; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Plasma. Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 1 mL portions of 2 M LiOH, wash with two 5 mL portions of water, wash with two 1 mL portions of EtOH:12 M HCl 90:10, wash with two 5 mL portions of water, wash with three 1 mL portions of buffer. 500 μL Plasma + 25 μL 10 nM isoproterenol + 500 μL buffer, mix, add to the SPE column, wash with two 5 mL portions of water, wash with 1 mL MeCN:water 50:50, elute with 300 μL 600 μM potassium ferricyanide in 600 mM KCl:MeCN 50:50, add 50 μL reagent to the eluate, heat at 37° for 40 min, cool in ice-water, inject a 100 μL aliquot. Urine. 10 μL Urine + 1 mL MeCN:500 mM KCl 60:40 + 10 μL 500 nM isoproterenol + 10 μL 75 mM potassium hexacyanoferrate(III) + 100 μL reagent, heat at 37° for 40 min, inject a 100 μL aliquot (J. Chromatogr. 1986, 380, 229). (Prepare buffer by mixing 8 volumes 250 mM LiOH in 200 mM phosphoric acid with 1 volume 200 mM phosphoric acid, pH 5.8. Prepare reagent by dissolving 212 mg 1,2-diphenylethylenediamine in 10 mL 100 mM HCl, pH 6.7.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 50:10:40 (Wash with MeCN:MeOH:water 50:10:40 for 15 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 345 em 485 (plasma), F ex 350 em 480 (urine)

CHROMATOGRAM
Retention time: 6
Internal standard: isoproterenol (8)
Limit of detection: 10 pM

OTHER SUBSTANCES
Extracted: epinephrine, norepinephrine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Mitsui,A.; Nohta,H.; Ohkura,Y. High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent, *J.Chromatogr.*, **1985**, *344*, 61–70.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 μL 4 M perchloric acid + 50 μL 1 μg/mL dihydroxybenzylamine in 0.1 M perchloric acid, centrifuge at 1500 g for 10 min. Remove 300 μL supernatant and centrifuge it at 1600 g through a 0.2 μm regenerated cellulose filter, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Biophase ODS + 50 × 4.6 Pelliguard LC-18
Column: 250 × 4.6 5 μm Biophase ODS or 250 × 4.6 Phase II ODS (both from Bioanalytical Systems)
Mobile phase: MeOH:buffer 5:95 (Buffer was 20 mM sodium citrate, 100 mM NaH_2PO_4, 0.15 mM, and 1.25 mM heptanesulfonic acid, pH 3.2.)
Column temperature: 28
Flow rate: 1-1.5
Injection volume: 20
Detector: E, Bioanalytical Systems LC-150 in dual-parallel mode, channel 1 700 mV 200 nA f.s. for levodopa and 3-O-methyldopa, channel 2 560 mV 10 nA f.s. for dopamine, carbidopa, and dihydroxyphenylacetic acid, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11.4
Internal standard: dihydroxybenzylamine (7)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: 3-O-methyldopa, carbidopa, levodopa, dihydroxyphenylacetic acid

KEY WORDS
plasma

REFERENCE
Cedarbaum,J.M.; Williamson,R.; Kutt,H. Simultaneous determination of levodopa, its metabolites and carbidopa in clinical samples, *J.Chromatogr.*, **1987**, *415*, 393–399.

SAMPLE
Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex pre-swollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. 1 mL Plasma + 30 μL 80 ng/mL N-methyldopamine, apply to column, wash with 5.5 mL water (A), elute with 3 mL 0.5 M perchloric acid. Collect eluate, add 2 mL 1.5 M pH 9.3 Tris buffer containing 60 mM EDTA, add 20 mg alumina, vortex for 2 min, discard supernatant, add 2 mL water to alumina, mix, centrifuge at 3000 g for 3 min, repeat water wash, remove as much water as possible, elute catecholamines from alumina with 100 μL 100 mM acetic acid with vortexing for 2 min, centrifuge, inject 25 μL aliquot of supernatant. (Wash water A contains levodopa, carbidopa, DOPAC, and O-methyldopa.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid
Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 13
Internal standard: N-methyldopamine (16)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: norepinephrine, epinephrine

KEY WORDS
plasma

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 1 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 1-octanol and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Discard the organic phase, thaw the aqueous phase and add it to 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA, shake, freeze in dry ice/acetone. Remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add it to 250 μL MeCN, 50 μL 1.75 M pH 7.05 bicine, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, keep at 20° in the dark, inject a 100 μL aliquot. (Buffer was 2 M pH 8.6 ammonia/ammonium chloride buffer containing 8.9 mM diphenylborate-ethanolamine complex and 13.4 mM EDTA. Stir buffer with 45 g/L activated alumina for 2 h before use. Wash 1-octanol with 80 mM acetic acid. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:8:50
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 5
Internal standard: α-methylnorepinephrine (3)
Limit of detection: 3 pg/mL

OTHER SUBSTANCES
Extracted: dihydroxybenzylamine, epinephrine, isoproterenol, norepinephrine

KEY WORDS
plasma; derivatization; comparison with electrochemical detection

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 17–28.

SAMPLE
Matrix: blood
Sample preparation: Pack a 65 × 15 SPE column with 50 mg WA-4 alumina (Sigma). Add 500 μL plasma to the SPE column, add 1 mL buffer, rotate for 15 min, wash three times with water (aspirating to dryness each time), centrifuge to dryness, add 200 μL 100 mM pH 1.2 perchloric acid, mix, let stand for 15 min, centrifuge the SPE column at 1000 g for 3 min, inject an aliquot of the effluent. (Buffer was 45 g Tris and 5 g EDTA in 200 mL water, pH adjusted to 8.6 with concentrated HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm reversed-phase
Column: 250 × 4.6 5 μm ODS Spherisorb
Mobile phase: Buffer contained 1.4% monochloroacetic acid, 0.47% NaOH, and 0.075% EDTA, finally pH adjusted to 3.0 with NaOH or monochloroacetic acid and 6 mg% sodium octylsulfate added.
Column temperature: 35
Flow rate: 1
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, TL-5 transducer with a glassy carbon electrode, +650 mV, 1 nA, Ag/AgCl reference electrode

OTHER SUBSTANCES
Extracted: epinephrine, norepinephrine

KEY WORDS
plasma; rabbit; human; SPE

REFERENCE
Ganhao,M.F.; Hattingh,J.; Hurwitz,M.L.; Pitts,N.I. Evaluation of a simple plasma catecholamine extraction procedure prior to high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.*, **1991**, *564*, 55–66.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 125 μL 2 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer

and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 75 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 125 μL 40 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm PhaseSep C18 ODS2

Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:4:76. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 40:60 for 3 min, go to 0:100 over 0.5 min, stay at 0:100 for another 4.5 min. (After the last sample flush column with 60 mL MeCN:MeOH:water 70:10:20.)

Flow rate: 1

Injection volume: 50-75

Detector: F ex 350 em 480

CHROMATOGRAM

Retention time: 5.5

Internal standard: α-methylnorepinephrine (2.5)

Limit of detection: 0.3-0.6 pg

OTHER SUBSTANCES

Simultaneous: norepinephrine, epinephrine, epinine

Interfering: α-methyldopa

KEY WORDS

plasma

REFERENCE

Boomsma,F.; Alberts,G.; van der Hoorn,F.A.J.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of free catecholamines and epinine and estimation of total epinine and dopamine in plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *574*, 109–117.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Plasma + 5 mg alumina + 10 μL 10 μM IS in 10 mM perchloric acid + 100 μL pH 8.7 Tris-HCl buffer, stir for 10 min, centrifuge at 3000 g for 1 min, discard the supernatant. Wash the alumina with two 500 μL portions of water, add 100 μL 100 mM perchloric acid, mix for 1 min, centrifuge at 3000 g for 1 min, inject a 50 μL aliquot of the supernatant. (Heat 20 g alumina (WA-4, Sigma) with 200 mL 2 M HCl at 100° for 1 h with gentle mixing, decant the supernatant, wash with twenty 200 mL portions of water, filter (Toyo Roshi No. 2 paper), dry at 120° overnight.)

HPLC VARIABLES
Column: 150 × 4.6 catecholpak (JASCO)
Mobile phase: MeCN:50 mM pH 3.20 potassium acetate:50 mM pH 3.20 potassium phosphate buffer 3:92.15:4.85 containing 1 mM sodium hexanesulfonate
Column temperature: 40
Flow rate: 0.5
Injection volume: 50
Detector: Chemiluminescence (Kenko filter Y-46) following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and the mixture flowed through a 15 m × 0.5 mm i.d. knitted PTFE coil at 80°. The effluent from the coil mixed with reagent 2 pumped at 1.4 mL/min and this mixture flowed to the detector. (Reagent 1 was 105 mM ethylenediamine (semiconductor grade) and 175 mM imidazole in MeCN:EtOH 90:10. Reagent 2 was 0.25 mM bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate (Wako), 150 mM hydrogen peroxide, and 110 mM trifluoroacetic acid in dioxane:ethyl acetate 50:50 (Caution! Dioxane is a carcinogen!).)

CHROMATOGRAM
Retention time: 22
Internal standard: 3,4-dihydroxybenzylamine (17)
Limit of detection: 1 fmole

OTHER SUBSTANCES
Extracted: epinephrine, norepinephrine

KEY WORDS
human; rat; plasma; SPE; post-column reaction

REFERENCE
Higashidate,S.; Imai,K. Determination of femtomole concentrations of catecholamines by high-performance liquid chromatography with peroxyoxalate chemiluminescence detection, *Analyst*, **1992**, *117*, 1863–1868.

SAMPLE
Matrix: blood
Sample preparation: Filter (Ultrafree-MC with 10000 molecular mass cut-off, Millipore) 100 μL plasma while centrifuging at 15000 g for 15 min. Mix 50 μL ultrafiltrate and 10 μL 140 ng/mL 3-methoxytyramine in Ringer solution, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Inertsil-2 ODS
Mobile phase: MeCN:THF:water 6:0.8:93.2 containing 0.48 g/L sodium 1-octanesulfonate, 2 g/L NaH_2PO_4, 8.82 g/L sodium citrate, 10 mg/L EDTA, and 1 mL/L diethylamine, pH adjusted to 3.2 with concentrated orthophosphoric acid.
Flow rate: 0.06
Injection volume: 5
Detector: E, Bioanalytical Systems BAS-4C, glassy carbon working electrodes, upstream +0.75 V, downstream +0.05 V (measuring electrode), Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.5
Internal standard: 3-methoxytyramine (11)
Limit of detection: 0.2-0.5 pg

OTHER SUBSTANCES
Extracted: norepinephrine, epinephrine, 3,4-dihydroxyphenylacetic acid, serotonin, 5-hydroxyindoleacetic acid, homovanillic acid

KEY WORDS
plasma; microbore; rat; ultrafiltrate

REFERENCE
Cheng,F.-C.; Yang,L.-L.; Kuo,J.-S.; Yang,M.C.M.; Yu,P.-C. Rapid assay of the monoamine content in small volumes of rat plasma, *J.Chromatogr.B*, **1994**, *653*, 9–16.

SAMPLE
Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass cut-off) 100 μL rat plasma while centrifuging at 15000 g for 15 min, mix 50 μL ultrafiltrate and 10 μL 140 ng/mL 3-methoxytyramine in Ringer solution containing 0.1 μM ascorbic acid in 100 mM HCl, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Inertsil-2 ODS
Mobile phase: MeCN:THF:water 6:0.8:93.2 containing 0.48 g/L sodium 1-octanesulfonate, 2 g/L NaH_2PO_4, 8.82 g/L sodium citrate, 10 mg/L EDTA, and 1 mL/L diethylamine, pH adjusted to 3.2 with orthophosphoric acid
Flow rate: 0.06
Injection volume: 5
Detector: E, Bioanalytical Systems BAS-4C, glassy carbon electrodes, +0.75 V upstream, +0.05 V downstream (measuring electrode), Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.5
Internal standard: 3-methoxytyramine (11)
Limit of detection: 0.2-0.5 pg

OTHER SUBSTANCES
Extracted: epinephrine, norepinephrine, serotonin, 5-hydroxyindoleacetic acid, homovanillic acid, 3,4-dihydroxyphenylacetic acid

KEY WORDS
plasma; rat; microbore; ultrafiltrate

REFERENCE
Cheng,F.-C.; Yang,L.-L.; Kuo,J.-S.; Yang,M.C.M.; Yu,P.-C. Rapid assay of the monoamine content in small volumes of rat plasma, *J.Chromatogr.B*, **1994**, *653*, 9–16.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 2 μg/mL 3,4-dihydroxybenzylamine in 0.4 M perchloric acid + 25 μL 70% perchloric acid, vortex 1 min, keep on ice for 1 min, store at -80°. Allow to thaw at +4°, vortex 1 min, centrifuge at 1200 g at +4° for 15 min. Remove 300 μL supernatant and add it to 200 μL 2 M pH 4.5 potassium citrate buffer, centrifuge at 1200 g at +4° for 10 min, inject 50 μL of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:MeCN:buffer 8:4:88 (Buffer was 50 mM pH 3.2 phosphate containing 3.5 mM heptanesulfonic acid and 0.05 mM EDTA.)
Flow rate: 1
Injection volume: 50
Detector: E, ESA Model 5100, Model 5020 guard cell +0.6 V, Model 5010 analytical cell, DET 1 +0.35 V, DET 2 -0.35 V, both DET 1 and DET 2 monitored

CHROMATOGRAM
Retention time: 11.83
Internal standard: 3,4-dihydroxybenzylamine (8.26)
Limit of detection: 19.6 ng/mL

OTHER SUBSTANCES
Simultaneous: 3-O-methyldopa, levodopa, L-DOPA methyl ester

KEY WORDS
plasma; rat; human

REFERENCE
Rondelli,I.; Acerbi,D.; Mariotti,F.; Ventura,P. Simultaneous determination of levodopa methyl ester, levodopa, 3-O-methyldopa and dopamine in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1994**, *653*, 17–23.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 69.05
Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids epinephrine, histamine, norepinephrine, taurine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Add 1 mL serum to 100 mg activated aluminum oxide suspended in 1 mL pH 8.7 Tris-HCl buffer, stir, let stand for 10 min. Discard the supernatant and wash the solid three times with 5 mL portions of water, wash the solid with 3 mL MeOH, dry under reduced pressure, elute with 3 mL 4 M acetic acid. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 90 μL water, inject a 10 μL aliquot. Urine. 5 mL Urine + 5.3 mL 2 M HCl, heat at 100° for 20 min, cool to room temperature, add 1 mL 50 mM disodium EDTA, adjust the pH to 8.5 with dilute ammonia, add 500 mg 200 mesh aluminum oxide (Wako), shake for 10 min, filter, wash the solid with 10 mL water, elute with 5 mL 300 mM acetic acid, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 3.6 10-25 μm Hitachi 3011 C resin
Mobile phase: 50 mM K_2HPO_4 containing 0.05% phosphoric acid
Column temperature: 45
Flow rate: 0.6
Injection volume: 10
Detector: F ex 383 em 486 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide in water pumped at 0.5 mL/min and with buffer pumped at 1 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° to the detector. (Buffer was 600 mM boric acid containing 750 mM KOH.)

CHROMATOGRAM
Retention time: 10
Limit of detection: 0.098 pmole

OTHER SUBSTANCES
Extracted: epinephrine, norepinephrine

KEY WORDS
post-column reaction; serum; SPE

REFERENCE
Honda,S.; Takahashi,M.; Araki,Y.; Kakehi,K. Postcolumn derivatization of catecholamines with 2-cyanoacetamide for fluorimetric monitoring in high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *274*, 45–52.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 150 μL Toyopak IC-SP S (sulfopropyl resin, H^+ form) SPE cartridge (Tosoh) with 10 mL water. Plasma. 700 μL Plasma + 50 μL 700 nM 3,4-dihydroxybenzylamine + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min. Add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Urine. Acidify urine collected over 24 h with 10 mL 6 M HCl. 500 μL Urine + 25 μL 10 μM 3,4-dihydroxybenzylamine + 25 μL 40 μM ferulic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4°

at 1000 g for 5 min, add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.; SPE

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)

Mobile phase: Gradient. A was buffer. B was MeCN:MeOH:buffer 8:12:80, pH 3.1. A:B 100:0 for 4 min, to 60:40 over 8 min, to 0:100 over 2 min, maintain at 0:100 for 16 min, return to initial conditions (step gradient), re-equilibrate for 20 min. Buffer was 60 mM pH 3.1 citric acid containing 32 mM Na_2HPO_4, 1.7 mM sodium hexanesulfonate, and 0.1 mM disodium EDTA (J. Chromatogr. 1989, 467, 237).

Flow rate: 1

Injection volume: 50

Detector: F ex 345 em 480 following post-column reaction. The column effluent passed through a Hitachi 655A electrochemical detector with carbon cloth electrodes; working electrode at +0.68 V versus reference electrode (200 mM equimolar mixture of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) containing 200 mM potassium nitrate and 200 mM KOH). The effluent from the electrochemical detector mixed with 20 mM meso-1,2-diphenylethylenediamine in 50 mM HCl pumped at 0.4 mL/min and with 1 M glycine containing 490 mM KOH and 3 mM potassium hexacyanoferrate(III) pumped at 0.4 mL/min. This mixture flowed through a 10 m × 0.47 mm ID coil at 80° to the detector (J. Chromatogr. 1989, 467, 237).

CHROMATOGRAM

Retention time: 18

Internal standard: 3,4-dihydroxybenzylamine (12.5)

Limit of detection: 2 nM (urine), 3 nM (plasma)

OTHER SUBSTANCES

Extracted: epinephrine, levodopa, metanephrine, 3-methoxytyramine, norepinephrine

KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Nohta,H.; Yamaguchi,E.; Ohkura,Y.; Watanabe,H. Measurement of catecholamines, their precursor and metabolites in human urine and plasma by solid-phase extraction followed by high-performance liquid chromatography with fluorescence derivatization, *J.Chromatogr.*, **1989**, *493*, 15–26.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 3 mL ice-cold MeOH:500 mM perchloric acid 98:2, centrifuge at 4° at 4000 g for 3 min. Remove 200 μL of the supernatant and add it to 100 μL 80 ng/mL N-methyldopamine, evaporate to dryness under vacuum, reconstitute in 200 μL mobile phase, inject a 5-20 μL aliquot. Urine. 1 mL Urine + 50 mL water, inject a 10 μL aliquot. (To deconjugate adjust pH to 1, flush with nitrogen, heat in a boiling water bath for 1 h, dilute with 50 mL water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeOH:13 mM sodium acetate containing 0.5 mM sodium 1-octanesulfonate and 0.5 mM disodium EDTA 14:86, pH 3.10

Flow rate: 1

Injection volume: 5-20

Detector: E, ESA Model 5100 A Coulochem, Model 5011 A analytical cell, first electrode +0.40 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 10
Internal standard: N-methyldopamine (11)

OTHER SUBSTANCES
Extracted: methyldopa, norepinephrine, epinephrine, dihydroxyphenylacetic acid, 3-O-methylmethyldopa, homovanilic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lucarelli,C.; Betto,P.; Ricciarello,G.; Grossi,G. High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) in human urine and plasma, *J.Chromatogr.*, **1991**, *541*, 285–296.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 30 μL 700 nM isoproterenol + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 10 μL 15 μM isoproterenol + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)
Flow rate: 0.8
Injection volume: 100
Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM
Retention time: 37
Internal standard: isoproterenol (60)
Limit of detection: 2-3 nM

OTHER SUBSTANCES
Extracted: epinephrine, levodopa, metanephrine, 3-methoxytyramine, norepinephrine, normetanephrine

KEY WORDS
post-column reaction; plasma; SPE

REFERENCE
Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or 1 mL urine + dihydroxybenzylamine + 20 mg Sigma WA4 alumina + 200 μL 1 M pH 8.6 Tris-EDTA buffer, mix for 10 min, discard plasma. Wash the alumina three times with 3 mL water and dry it. Add 125 μL 500 mM phosphoric acid, after 1 min inject a 100 μL aliquot. (Ann. Clin. Biochem. 1985, 22, 194-203)

HPLC VARIABLES
Column: 250 × 4.5 5 μm Ultratechsphere
Mobile phase: Per liter 75 mmol citric acid, 58.5 mmol NaH_2PO_4, 0.2 mmol disodium EDTA, and 4.4 mmol heptanesulfonic acid, pH adjusted to 3.4, made up to a final volume of 2 L, add 200 mL MeOH
Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem conditioning cell +0.35 V, first electrode +0.05 V, second electrode -0.35 V

CHROMATOGRAM
Retention time: 15.89
Internal standard: dihydroxybenzylamine (10.53)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: levodopa, metanephrine, norepinephrine, 3-methoxytyrosine, normetanephrine, dihydroxyphenylacetic acid, epinephrine

KEY WORDS
plasma

REFERENCE
Dutton,J.; Copeland,L.G.; Playfer,J.R.; Roberts,N.B. Measuring L-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with L-dopa and a dopa decarboxylase inhibitor, *Clin.Chem.*, **1993**, *39*, 629–634.

SAMPLE
Matrix: dialysate
Sample preparation: Wash the dialysis probe with artificial CSF at 1.5 μL/min (Artificial CSF was 145 mM Na^+, 2.7 mM K^+, 1.0 mM Mg^{++}, and 1.2 mM Ca^{++}, adjusted to pH 7.4 ± 0.2 with phosphate buffer.). Inject a 20 μL aliquot of the dialysate sample.

HPLC VARIABLES
Column: 80 × 4.6 3 μm HR-80 C18 (ESA, Bedford, MA)
Mobile phase: MeCN:MeOH:buffer 15:20:65 (Buffer was 75 mM NaH_2PO_4 containing 1 mM sodium dodecyl sulfate and 100 μM EDTA, adjusted to pH 5.7 with NaOH.)
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5014, dual electrode analytical cell, first electrode -175 mV, second electrode +175 mV

CHROMATOGRAM
Limit of detection: 100 nM

KEY WORDS
rat; stratium; brain; microdialysis

REFERENCE
Balcioglu,A.; Wurtman,R.J. Dexfenfluramine enhances striatal dopamine release in conscious rats via a serotoninergic mechanism, *J.Pharmacol.Exp.Ther.*, **1998**, *284*, 991–997.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 5 μL aliquot of dialysate.

HPLC VARIABLES
Column: 100 × 1 3 μm SepStik (BioAnalytical Systems, West Lafayette, IN)
Mobile phase: THF:50 mM NaH_2PO_4 24:76 containing 0.2 mM disodium EDTA, 3.7 mM 1-decanesulfonic acid, and 4.9 mM triethylamine, pH 5.5
Flow rate: 0.023
Injection volume: 5
Detector: E, (EG & G Princeton Applied, USA), Model MP 1304, glassy carbon electrode, first at +700 mV, second at 0 mV, Ag/AgCl reference electrode (BioAnalytical Systems, Model RE4); UV 225

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 1 nM

OTHER SUBSTANCES
Simultaneous: cocaine (UV 225)

KEY WORDS
rat; brain; microdialysis; pharmacokinetics; microbore

REFERENCE
Parsons,L.H.; Kerr,T.M.; Weiss,F. Simple microbore high-performance liquid chromatographic method for the determination of dopamine and cocaine from a single in vivo brain microdialysis sample, *J.Chromatogr.B*, **1998**, *709*, 35–45.

SAMPLE
Matrix: dialysate
Sample preparation: Mix 30 μL dialysate + 5 μL 200 mM perchloric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 3 μm HR-80 C18 (ESA)
Mobile phase: MeCN:MeOH:buffer 15:13:72 (Buffer was 75 mM NaH_2PO_4, 1.5 mM sodium dodecyl sulfate, 100 μL/L triethylamine, and 20 μm EDTA, adjusted to pH 5.6.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5014 Microdialysis Cell with E1 -175 mV and E2 +175 mV, Pd reference electrode, Model 5020 Guard Cell EGC +300 mV

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 400 fg

OTHER SUBSTANCES
Extracted: norepinephrine, epinephrine, serotonin

KEY WORDS
rat; brain; pharmacokinetics; use peek tubing and sample loop

REFERENCE
Gariepy,K.C.; Bailey,B.; Yu,J.; Maher,T.; Acworth,I.N. Simultaneous determination of norepinephrine, dopamine, and serotonin in hippocampal microdialysis samples using normal bore high performance liquid chromatography: Effects of dopamine receptor agonist stimulation and euthanasia, *J.Liq.Chromatogr.*, **1994**, *17*, 1541–1556.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:buffer 13:87 (Buffer was 0.01% acetic acid containing 0.001% sodium octanesulfonate.)
Flow rate: 2.5
Detector: UV 280

CHROMATOGRAM
Internal standard: benzoic acid

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: k' 5.5
Limit of detection: 7 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, norepinephrine, phenylephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE

Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.6 5 μm Lichrospher RP 18 end-capped
Mobile phase: MeCN:acetic acid:50 mM potassium phosphate buffer 20:10:70
Injection volume: 20
Detector: UV 280

OTHER SUBSTANCES

Simultaneous: dobutamine

KEY WORDS

injections; 5% dextrose; stability-indicating

REFERENCE

Sautou-Miranda,V.; Gremeau,I.; Chamard,I.; Cassagnes,J.; Chopineau,J. Stability of dopamine hydrochloride and of dobutamine hydrochloride in plastic syringes and administration sets, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 186–193.

SAMPLE

Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES

Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 70 mV

CHROMATOGRAM

Retention time: 5.57
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: apomorphine, hydralazine, isoproterenol, methoxamine, morphine, norepinephrine, phenylephrine

KEY WORDS

rat; derivatization

REFERENCE

Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE

Matrix: solutions

Sample preparation: Dilute a few μL of a <1 mM solution to 20 μL with 50 mM pH 8.0 phosphate or borate buffer, add 10 μL 2 mg/mL (?) fluorescamine in acetone with vigorous shaking, inject an aliquot.

HPLC VARIABLES

Column: 500 × 3 Hitachi 3011 gel glass column

Mobile phase: MeOH:100 mM pH 8.0 Tris-HCl buffer 70:30

Flow rate: 0.72

Detector: F primary filter Corning No. 7-51, secondary filter No. 4-7116

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Simultaneous: norepinephrine

Also analyzed: 3-methoxytyramine, normetanephrine

KEY WORDS

derivatization

REFERENCE

Imai,K. Fluorimetric assay of dopamine, norepinephrine and their 3-O-methyl metabolites by using fluorescamine, *J.Chromatogr.*, **1975**, *105*, 135–140.

SAMPLE

Matrix: solutions

Sample preparation: 200 μL 5 μg/mL Amine solution + 300 μL 100 mM pH 8.0 phosphate buffer + 300 μL 200 μg/mL fluorescamine in acetone + 200 μL water, mix, saturate with NaCl, add 500 μL ethyl acetate, shake for 1 min, inject a 50 μL aliquot of the organic phase.

HPLC VARIABLES

Column: 250 × 2 10 μm LiChrosorb Si 60-10

Mobile phase: Benzene:dioxane:acetic acid 76:22:2 (Caution! Benzene and dioxane are carcinogens!)

Flow rate: 0.5

Injection volume: 50

Detector: F ex 325-385 (filter) em 451

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: norepinephrine

KEY WORDS

derivatization; normal phase

REFERENCE

Schwedt,G. Hochdruck-Flüssigkeits-chromatographische Analyse der Katecholamine Dopamin und Noradrenalin als Fluorescaminderivate, *J.Chromatogr.*, **1976**, *118*, 429–432.

SAMPLE

Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 20 μL aliquot.

HPLC VARIABLES

Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 20:80
Flow rate: 1.6-2.0
Injection volume: 20
Detector: F ex 285 em 315

CHROMATOGRAM

Retention time: 4.08

OTHER SUBSTANCES

Interfering: epinephrine

REFERENCE

Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclam-

ide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Prepare a 70 × 5 SPE column of Sephadex G 10 in a Pasteur pipette, wash with 3 mL 20 mM ammonia and 3 mL 10 mM formic acid, let stand for 10 days. Homogenize up to 150 mg rat brain in 1 mL 100 mM perchloric acid, centrifuge at 4000 g at 4° for 15 min, add 500 μL of the supernatant to the SPE column, wash with 2.5 mL 10 mM formic acid, elute with 1 mL 10 mM formic acid followed by 1.5 mL 5 mM Na_2HPO_4, inject an aliquot of the eluate.

HPLC VARIABLES

Column: Nucleosil 5 C18

Mobile phase: pH 5.5 Buffer prepared from 200 mM Na_2HPO_4 and 100 mM citric acid

Flow rate: 0.8

Injection volume: 200

Detector: E, rotating disc electrode, 500 mV

CHROMATOGRAM

Retention time: 8

Limit of detection: 0.05 nmole/g

OTHER SUBSTANCES

Extracted: levodopa, uric acid

KEY WORDS
rat; brain; SPE

REFERENCE
Westerink,B.H.C.; Mulder,T.B.A. Determination of picomole amounts of dopamine, noradrenaline, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid in nervous tissue after one-step purification on Sephadex G-10, using high-performance liquid chromatography with a novel type of electrochemical detection, *J.Neurochem.*, **1981**, *36*, 1449–1462.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (glass potters) with 5 volumes of 100 mM perchloric acid containing 1.9 mM sodium bisulfite, centrifuge at 10000 g at 4° for 30 min. Filter (0.22 μm) the supernatant, add dihydroxybenzylamine, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Hypersil H5 ODS
Mobile phase: EtOH:buffer 2:98 (Buffer was 13.8 g NaH_2PO_4, 60 mg disodium EDTA, and 20 mg 1-octanesulfonic acid in 1 L water, pH adjusted to 3.70 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-20
Detector: E, Unicam PU 4022, 70 mV

CHROMATOGRAM
Retention time: 22
Internal standard: dihydroxybenzylamine

OTHER SUBSTANCES
Extracted: norepinephrine, epinephrine

KEY WORDS
adrenal; fetal

REFERENCE
Garciá,J.C.; Blanco,L.; McPherson,M.; Leiva,A.; Maciás,R. High-performance liquid chromatographic determination of norepinephrine, epinephrine and dopamine in human foetal adrenal gland, *J.Chromatogr.B*, **1994**, *656*, 77–80.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron PT 3000) brain tissue in 1 mL cold 100 mM perchloric acid containing 1 mg/mL sodium bisulfite and 1 mM EDTA, centrifuge at 12000 rpm for 2 min. Filter (0.22 μm cellulose acetate) and inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Biophase ODS II C18 (BAS)
Mobile phase: MeOH:buffer 12.5:87.5, pH 3.7 (Buffer was 100 mM citric acid/sodium acetate containing 1 mM EDTA and 4.5-5.3 sodium heptanesulfonate.)
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Extracted: 3-O-methyl-L-DOPA

Noninterfering: levodopa

KEY WORDS
rat; brain; 11C labelled; DOPAC; homovanillic acid

REFERENCE
Lindner,K.-J.; Hartvig,P.; Tedroff,J.; Ljungström,A.; Bjurling.P.,; Långström,B. Liquid chromatographic analysis of brain homogenates and microdialysates for the quantification of L-[β-^{11}C]DOPA and its metabolites for the validation of positron emission tomography studies, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 361–367.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron PT 10-35) 50 mg brain tissue with 500 μL 10 μM disodium EDTA in 100 mM perchloric acid containing isoproterenol, centrifuge at 4° at 4000 rpm for 10 min, filter (dismic-3cp cellulose acetate, 0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: Supelcosil LC-18-DB
Column: Supelcosil LC-18-DB
Mobile phase: MeOH:10 mM pH 4.4 citrate buffer 10:90 containing 10 μM disodium EDTA and 0.5 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 10
Detector: E, Eicom ECD-100, +0.7 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 27
Internal standard: isoproterenol (45)

OTHER SUBSTANCES
Extracted: 3,4-dihydroxyphenylacetic acid, homovanillic acid

KEY WORDS
rat; brain; pharmacokinetics

REFERENCE
Sato,S.; Koitabashi,T.; Koshiro,A. Pharmacokinetic and pharmacodynamic studies of chlorpromazine in rats: Effect of chlorpromazine on dopamine and dopamine metabolite concentration in rat striatum, *Biol.Pharm.Bull.*, **1995**, *18*, 990–995.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine with 1% (v/v) 6 M HCl. 6-10 mL Swine urine or 1-2.5 mL rat urine, centrifuge at 4000 g for 30 min, add 200 ng 3,4-dihydroxybenzylamine hydrobromide and 15 mL 1g/L EDTA, adjust to pH 6.45-6.55 with HCl or NaOH. Add the mixture to a cation-exchange resin SPE cartridge (Bio-Rad), wash twice with 10 mL water and with 5 ml water, elute with 8 mL 10 g/L boric acid. Dilute boric acid eluate with an equal volume of mobile phase, inject a 60 μL aliquot. (Procedure for determining methoxycatecholamines is also described.); SPE

HPLC VARIABLES
Column: 150 × 4.6 5 μm Kromasil C8
Mobile phase: MeOH:buffer 15:85 (Mobile phase was 300 mL MeOH, 1.5 mL 200 mg/mL 1-octanesulfonic acid, 100 mL 1 M sodium acetate, and about 1 L water. The pH was adjusted to pH 3.8 with citric acid and made up to 2 L with water.)

Flow rate: 0.6
Injection volume: 60
Detector: E, Bioanalytical Systems, glassy carbon electrode + 650 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 14.23
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (10.31)
Limit of detection: 40 pg

OTHER SUBSTANCES
Extracted: epinephrine, norepinephrine

KEY WORDS
pig; rat; SPE; pharmacokinetics

REFERENCE
Hay,M.; Mormède,P. Determination of catecholamines and methoxycatecholamines excretion patterns in pig and rat urine by ion-exchange liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1997**, *703*, 15–23.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 2 mL water + 1 mL 500 μM EDTA in 1 mM HCl + 1 mL 2 M pH 8.5 phosphate buffer + 20 μL 100 μM 3,4-dihydroxybenzylamine + 50 mg alumina, vortex for 3 min, discard the supernatant, wash the alumina 3 times with 5 mL portions of water, elute by washing the alumina twice with 200 μL portions of 100 mM phosphoric acid for 30 s each time. Combine the acidic layers and add them to 560 μL 400 mM pH 9.0 borate buffer, add 20 μL 50 mM NaCN, add 20 μL 5 mM naphthalene-2,3-carboxaldehyde in MeOH, mix thoroughly, let stand at room temperature for 20 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-120T (Toyo Soda)
Mobile phase: MeCN:THF:10 mM pH 2.5 phosphate buffer 38:6:56
Flow rate: 1
Injection volume: 5
Detector: F ex 420 em 483

CHROMATOGRAM
Retention time: 10
Internal standard: 3,4-dihydroxybenzylamine (15.5)
Limit of detection: 20 fmole

OTHER SUBSTANCES
Extracted: norepinephrine

KEY WORDS
derivatization; SPE

REFERENCE
Kawasaki,T.; Higuchi,T.; Imai,K.; Wong,O.S. Determination of dopamine, norepinephrine, and related trace amines by prechromatographic derivatization with naphthalene-2,3-dicarboxaldehyde, *Anal.Biochem.*, **1989**, *180*, 279–285.

SAMPLE
Matrix: urine

Sample preparation: Add disodium EDTA and sodium metabisulfite to urine. 100 μL Urine + 2 mL water, vortex, add 1 mL reagent 1, add 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add it to 1 mL reagent 1 and 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add 100 μL Bicine buffer, 250 μL MeCN, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, vortex, add 20 μL 20 mM potassium ferricyanide, vortex, heat at 37° for 40 min, cool to room temperature, inject a 100 μL aliquot. (Prepare reagent 1 by dissolving 214 g ammonium chloride and 10 g disodium EDTA in 2 L water, adjust pH to 8.3-8.5 with concentrated ammonium hydroxide, add 4.0 g diphenylborate-ethanolamine complex, stir for several hours until a clear solution is obtained. Prepare reagent 2 by dissolving 2.5 g tetraoctylammonium bromide and 10 mL n-octanol (saturated with acetic acid) in 1 L n-heptane. Prepare Bicine buffer by dissolving 14.3 g Bicine (N,N-bis(2-hydroxyethyl)glycine) and 359 mg anhydrous sodium acetate in 45 mL water, stir overnight until dissolved, adjust pH to 7.30 with concentrated NaOH, make up to 50 mL with water. Note that concentration of 1,2-diphenylethylenediamine is not given in paper. Other authors have used 100 mM (J.Chromatogr. 1989, 487, 17; 1992, 574, 109; 1992, 583, 236).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:10:50. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 50:10:40. A:B 75:25 for 1 min, to 10:90 over 7 min, return to initial conditions over 1 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 418 (cutoff filter)

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Extracted: epinephrine, norepinephrine
Simultaneous: isoproterenol

KEY WORDS

derivatization; protect from light

REFERENCE

Moleman,P.; van Dijk,J. Determination of urinary norepinephrine and epinephrine by liquid chromatography with fluorescence detection and pre-column derivatization, *Clin.Chem.*, **1990**, *36*, 732–736.

SAMPLE

Matrix: urine
Sample preparation: Add 10-15 mL 6 M HCl to a 24 h volume of urine. 2 mL 3 M Tris buffer containing 30 mM EDTA + 500 μL 10 μM dihydrobenzylamide in 100 mM perchloric acid + 2 mL 3 M tris buffer containing 30 mM EDTA + 100 μL 5 M NaOH + 4 mL acidified urine, mix, add to a 1 mL SPE column containing 200 mg alumina (70-230 mesh-ASTM (Touzart et Matignon) at 3 mL/min, wash with 9 mL water at 12 mL/min, force through 0.5 mL air, elute with 1 mL 150 mM perchloric acid at 0.75 mL/min, mix eluate, inject a 100 μL aliquot. (The pH of the Tris buffer is such that the pH of the mixture applied to the SPE column is 7.75-8.0.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 100/C18
Mobile phase: MeOH:buffer 36:64 (Buffer was 75 mM NaH_2PO_4, 0.15 mM EDTA, and 6 mM sodium heptanesulfonate, pH 3.96.)
Flow rate: 1.25
Injection volume: 100
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 12.5
Internal standard: dihydrobenzylamide hydrobromide (9.6)
Limit of detection: 30 nM

OTHER SUBSTANCES
Extracted: norepinephrine, epinephrine
Simultaneous: levodopa, methyldopa

KEY WORDS
SPE

REFERENCE
Said,R.; Robinet,D.; Barbier,C.; Sartre,J.; Huguet,C. Fully automated high-performance liquid chromatographic assay for the analysis of free catecholamines in urine, *J.Chromatogr.*, **1990**, *530*, 11–18.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 100 μL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA + 25 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 25 mg alumina + 1 mL 2 M pH 8.6 Tris-HCl buffer in a microfilter tube (Centrex, Schleicher & Schuell), vortex 5 min, allow to stand for 10 min, filter off water, wash with 5 mL water, add 5 mL water, centrifuge at 3000 g, vortex with 400 μL 0.2 M perchloric acid containing 11 mM disodium EDTA and 0.4 M sodium metabisulfite, centrifuge at 9000 g for 5 min, inject 50 μL of filtrate. (Stabilize each 10 mL urine sample immediately with 0.5 mL 0.1 M HCl and 1 mL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA.)

HPLC VARIABLES
Guard column: 40 × 4.6 Bio-Sil ODS-10 (Bio-Rad)
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:water 22.5:77.5 containing 20 mM citric acid, 20 mM Na_2HPO_4, 4 mM sodium octanesulfonate, and 0.05 mM disodium EDTA, pH adjusted to 2.74 ± 0.01 with 2 M citric acid
Column temperature: 40
Injection volume: 50
Detector: E, BAS LC-4B, 0.54 V vs Ag/AgCl, 50 nA full scale

CHROMATOGRAM
Retention time: 8.5
Internal standard: α-ethyldopa (14)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: levodopa, carbidopa

KEY WORDS
SPE

REFERENCE

Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

SAMPLE

Matrix: urine

Sample preparation: 100 μL Urine + 125 μL 218.6 nM α-methylnorepinephrine in 10 mM HCl + 1 mL 10 mM HCl + 1 mL reagent + 5 mL 4.6 mM tetraoctylammonium bromide in n-heptane:1-octanol 99:1, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol saturated with 80 mM acetic acid and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 1 mL 10 mM HCl to the aqueous layer, add 2 mL 1-octanol saturated with 80 mM acetic acid, add 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 200 μL MeCN and 50 μL buffer to the aqueous layer, add 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, inject a 50 μL aliquot. (Reagent was 8.9 mM diphenylborate-ethanolamine complex in 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA. Buffer was 1.75 M pH 7.05 bicine in water containing 1% EDTA. Recrystallize 1,2-diphenylethylenediamine from toluene: light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)

Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate 40:8:50 (At the end of the day flush column with 60 mL MeCN:MeOH:water 70:10:20.)

Flow rate: 1

Injection volume: 50

Detector: F ex 350 em 480

CHROMATOGRAM

Retention time: 5

Internal standard: α-methylnorepinephrine (Janssen, Beerse, Belgium) (3)

Limit of quantitation: 24.8 nM

OTHER SUBSTANCES

Extracted: epinephrine, norepinephrine

KEY WORDS

derivatization; protect from light

REFERENCE

van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Improved measurement of urinary catecholamines by liquid-liquid extraction, derivatization and high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1991**, *563*, 348–355.

SAMPLE

Matrix: urine

Sample preparation: Condition a 100 mg Bakerbond C-18 SPE cartridge with 2 mL MeOH and 2 mL buffer I. Heat urine at 50°, centrifuge, remove a 500 μL aliquot and add it to 1 mL buffer II (?), add 20 μL 860 ng/mL dihydroxybenzylamine, shake for 5 min, add a 1 mL aliquot to the SPE cartridge, wash with 2 mL buffer I, wash with 1 mL MeOH: buffer I 50:50, wash with 500 μL water, elute with 2 mL 1 M acetic acid, inject a 20 μL aliquot. (Buffer I was 200 mM ammonium chloride containing 0.05% EDTA and 0.4% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1. Buffer II was 2 M ammonium

chloride containing 0.5% EDTA and 1.2% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1.); SPE

HPLC VARIABLES

Column: 150 × 3 5 μm Separon SGX C-18 (Tessek)
Mobile phase: MeOH:buffer 5:95-7:93 (Buffer was 50 mM pH 3.0 ± 0.1 phosphate buffer containing 50 mM EDTA, 1 mM sodium octanesulfonate, and 1 mM NaCl.)
Injection volume: 20
Detector: E, AMOR 400 mV (SunChrom)

CHROMATOGRAM

Internal standard: dihydroxybenzylamine
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: epinephrine, norepinephrine

KEY WORDS

SPE

REFERENCE

Brandsteterova,E.; Krajnak,K.; Skacani,I. HPLC analysis of urinary catecholamines using affinity SPE procedure, *Pharmazie*, **1995**, *50*, 825–826.

SAMPLE

Matrix: urine
Sample preparation: Adjust urine to pH 3.0 with 6 M HCl, centrifuge at 1600 g for 10 min. 900 μL Supernatant + 100 μL 2.5 μM N-methyldopamine, mix, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 35 × 4 TSK-precolumn-CA 2 (Tosoh); B 150 × 4.6 mixed mode (C18/cation exchange) (Alltech)
Mobile phase: A 15 mM pH 6.0 citric acid/trisodium citrate buffer; B 200 mM pH 6.0 citric acid/trisodium citrate buffer
Column temperature: 35
Flow rate: 0.7
Injection volume: 100
Detector: E, Mitsubishi 8 channel, 30 mV

CHROMATOGRAM

Retention time: 15
Internal standard: N-methyldopamine (30 mV) (20)
Limit of detection: 1 nM

OTHER SUBSTANCES

Extracted: epinephrine (150 mV), metanephrine (380 mV), 3-methoxytyramine (340 mV), norepinephrine (150 mV), normetanephrine (380 mV), serotonin (150 mV)

KEY WORDS

column-switching

REFERENCE

Mashige,F.; Matsushima,Y.; Miyata,C.; Yamada,R.; Kanazawa,H.; Sakuma,I.; Takai,N.; Shinozuka,N.; Ohkubo,A.; Nakahara,K. Simultaneous determination of catecholamines, their basic metabolites and serotonin in urine by high-performance liquid chromatography using a mixed-mode column and an eight-channel electrochemical detector, *Biomed.Chromatogr.*, **1995**, *9*, 221–225.

SAMPLE

Matrix: urine

Sample preparation: Condition a 10 × 2 20 mg 15-25 μm PLRP-S polymer-based SPE cartridge (Spark Holland) with 1 mL MeOH, with 0.5 mL water, and with 1.5 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA. Collect 24 h urine with 10 mL 6 M HCl, final pH 1-3. Dilute 4-fold with buffer. Inject a 200 μL aliquot onto the SPE cartridge, wash with 1 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA, wash with 250 μL MeOH:200 mM pH 8.5 ammonia/ammonium chloride buffer 20:80, wash with water at 1 mL/min for 2.25 min. Elute the contents of the SPE cartridge onto column A with the mobile phase for 30 s then remove the SPE cartridge from the circuit, elute column A with mobile phase onto column B, after 1.25 min elute column A to waste with mobile phase and elute column B with mobile phase, monitor the effluent from column B. (Buffer was 2 M pH 8.5 ammonia/ammonium chloride containing 0.5% EDTA, 0.1% diphenylborate, and 18 ng/mL dihydroxybenzylamine.); SPE

HPLC VARIABLES

Column: A 30 × 4.6 C18 (Brownlee); B 250 × 4.6 5 μm Ultrasphere IP C18

Mobile phase: MeCN:MeOH:buffer 15:8:100, apparent pH adjusted to 3.2 with 1.5 M orthophosphoric acid (Buffer was 50 mM KH_2PO_4 containing 1 mM sodium heptane sulphate and 0.07 mM EDTA.)

Flow rate: 0.8

Injection volume: 200

Detector: E, ESA Model 5100A, Model 5021 conditioning cell, Model 5011 analytical cell, oxidizing electrode +350 mV, screen electrode +100 mV, quantifying electrode -300 mV

CHROMATOGRAM

Retention time: 10

Internal standard: dihydroxybenzylamine (8)

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Extracted: norepinephrine, epinephrine

KEY WORDS

SPE; column-switching

REFERENCE

Pastoris,A.; Cerutti,L.; Sacco,R.; De Vecchi,L.; Sbaffi,A. Automated analysis of urinary catecholamines by high-performance liquid chromatography and on-line sample pretreatment, *J.Chromatogr.B*, **1995**, *664*, 287–293.

SAMPLE

Matrix: urine

Sample preparation: Acidify urine to pH 2.0-3.5 with 5 M HCl, centrifuge at 7000 g for 10 min, inject a 10-500 μL aliquot on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A nitrophenylboronic acid modified copolymer (U.S. Patent 4 767 529 (Chem.Abs. 1988, 108, 71698t)); B 53 × 4.6 1.5 μm MICRA NPS RP-18 (MICRA Scientific, Northbrook)

Mobile phase: A 20 mM $(NH_4)_2HPO_4$ containing 10 mM EDTA, adjusted to pH 8.7 with 25% ammonia solution; B 10 mM NaH_2PO_4 containing 0.1 mM dodecanesulfonic acid, adjusted to pH 2.5 with orthophosphoric acid

Flow rate: A 0.5; B from 0.2 to 0.5 over 2 min, maintain at 0.5

Injection volume: 10-500

Detector: F ex 275 em 330

CHROMATOGRAM

Retention time: 12

Limit of detection: 15.61 pmole

Limit of quantitation: 36.81 pmole

OTHER SUBSTANCES

Extracted: epinephrine, norepinephrine

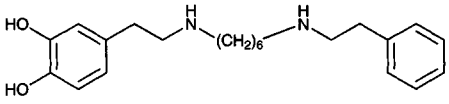
KEY WORDS

column-switching

REFERENCE

Rudolphi,A.; Boos,K.-S.; Seidel,D. Coupled-column HPLC analysis of free urinary catecholamines using restricted access affinity precolumn and micro-particulate nonporous silica analytical column, *Chromatographia*, **1995**, *41*, 645–650.

Dopexamine



Molecular formula: $C_{22}H_{32}N_2O_2$
Molecular weight: 356.51
CAS Registry No.: 86197-47-9, 86484-91-5 (HCl)
Merck Index: 3482
Lednicer No.: 4 22

SAMPLE

Matrix: blood
Sample preparation: Add 5 mL blood immediately to 250 mg sodium metabisulfite and dipotassium EDTA, freeze. 500 μL Blood + 500 μL 10 mM perchloric acid containing 1 mM disodium EDTA and 5 mM sodium metabisulfite + 1 mL 400 mM perchloric acid, vortex, shake horizontally at 300 oscillations/min for 10 min, centrifuge at 1800 g for 10 min, inject a 200 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.9 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 49:51 (Buffer was 4.04 g/L sodium 1-heptanesulfonate containing 1 mM disodium EDTA, 10 mL/L orthophosphoric acid, and 22.5 mL/L diisopropylamine. A 250 × 4.6 25-40 μm silica column was placed before the injector.)
Column temperature: 37-43
Flow rate: 1.2
Injection volume: 200
Detector: E, Metrohm 641 electrometer, Metrohm 656 detector cell, glassy carbon working electrode +0.55 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 7
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, acetaminophen, allopurinol, amiloride, amiodarone, amoxicillin, aspirin, atenolol, atropine, captopril, cefuroxime, clindamycin, colchicine, cyclizine, diazepam, diclofenac, dicloxacillin, digoxin, dipyridamole, dobutamine, dopamine, epinephrine, furosemide, gentisic acid, glyburide, heparin, hydrochlorothiazide, isoproterenol, isosorbide dinitrate, lidocaine, meperidine, metoclopramide, metolazone, mexiletine, netilmicin, nifedipine, pancuronium, procainamide, quinidine, ranitidine, salicylic acid, spironolactone, warfarin

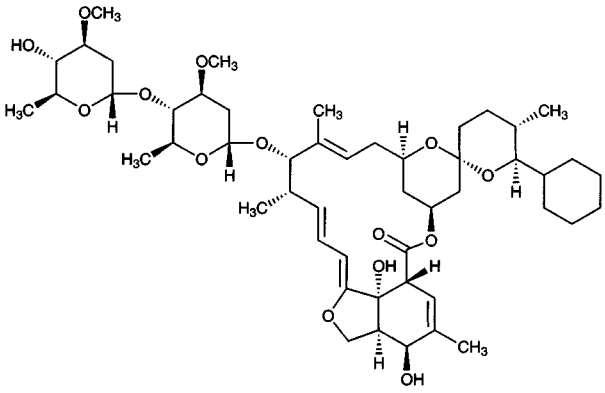
KEY WORDS

whole blood; detector temp 37-43°; pharmacokinetics

REFERENCE

Baker,P.R.; Gardner,J.J.; Lockley,W.J.S.; Wilkinson,D. Determination of dopexamine hydrochloride in human blood by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1995**, *667*, 283–290.

Doramectin



Molecular formula: $C_{50}H_{74}O_{14}$
Molecular weight: 899.13
CAS Registry No.: 117704-25-3
Merck Index: 3483

SAMPLE

Matrix: blood

Sample preparation: Condition a 96-well SPE block (Microlute) packed with 50 mg IST-C18 per well with 1 mL MeOH and 1 mL water. Mix 1 mL plasma with 500 µL 20 ng/mL IS solution . Add the mixture to the 96-well SPE block, draw it through the cartridge using a pulsed vacuum (100 ms on, 5000 ms off, total cycle time 20 min), wash with 1 mL water using vacuum. Elute with 1 mL MeOH under vacuum, evaporate at 60° under air in situ using a 96-place Techne sample concentrator. Add 100 µL 50% triethylamine (v/v) in MeCN, shake gently, add 150 µL 33% trifluoroacetic anhydride in MeCN, shake gently. Concentrate the sample to 100 µL, add 250 µL 2 M ammonia solution in MeOH, shake gently, concentrate the sample to ca. 100 µL, add 100 µL MeCN, inject a 100 µL aliquot. (Prepare IS solution as follows. Prepare a 30 µg/mL solution of IS in MeOH. Dilute with MeCN:water 30:70 to an IS concentration of 20 ng/mL. Centrifuge plasma at 2500 g for 10 min prior to analysis.)

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb S5C8
Mobile phase: MeCN:THF:water 67.5:15:17.5
Flow rate: 1
Injection volume: 100
Detector: F ex 360 em 470

CHROMATOGRAM

Retention time: 11.5
Limit of quantitation: 500 pg/mL

KEY WORDS

cow; plasma; derivatization; SPE

REFERENCE

Harrison,A.C.; Walker,D.K. Automated 96-well solid phase extraction for the determination of doramectin in cattle plasma, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 777–783.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 3 mL 500 mg Isolute C8 end-capped SPE cartridge (International Sorbent Technologies) with 5 mL MeCN and 5 mL MeCN:water 1:2 containing 0.1% triethylamine. Condition a 3 mL 500 mg Isolute silica SPE cartridge (International Sorbent Technologies) with 5 mL ethyl acetate:hexane 40:60. Homogenize (Tissuemizer) 5 g tissue in 25 mL MeCN, centrifuge at 3000 rpm for 5 min, decant supernatant into 50 mL water and 75 µL triethylamine. mix, pass through the C8 SPE cartridge at 2 mL/min, discard eluate, dry column under vacuum for 5 min, elute with 5 mL MeCN. Dry

the eluate under a stream of nitrogen at 50-55°, resolubilize dry residues in 5 mL of ethyl acetate:hexane 40:60, vortex briefly, pass through the silica SPE cartridge at 2 mL/min. Rinse the reservoir with 5 mL ethyl acetate:hexane 40:60, add the rinse to the SPE cartridge. Dry the SPE cartridge for 5 min, elute with 5 mL MeOH:ethyl acetate 50:50, dry the eluate under a stream of nitrogen at 50-55°. Reconstitute the extract with 200 μL of fresh methylimidazole:MeCN 50:50, vortex briefly, add 300 μL of fresh trifluoroacetic anhydride:MeCN 1:2, vortex briefly, dry under a stream of nitrogen at 50-55° for 15 min. Add 500 μL MeOH:ammonium acetate:molecular sieves 4:1:1), vortex briefly, dry under a stream of nitrogen at 50-55°. Add 1 mL MeCN, vortex thoroughly, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil ODS (C18)
Mobile phase: MeCN-water 90:10
Column temperature: 65
Flow rate: 1.0
Injection volume: 50
Detector: F ex 272 em 465

CHROMATOGRAM

Retention time: 10
Limit of detection: 0.25 ppb

OTHER SUBSTANCES

Extracted: ivermectin

KEY WORDS

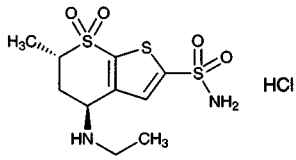
SPE; derivatization; salmon; muscle

REFERENCE

Rupp,H.S.; Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Long,A.R. Determination of ivermectin in salmon muscle tissue by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1998**, *81*, 549–553.

Dorzolamide hydrochloride

Molecular formula: $C_{10}H_{16}N_2O_4S_3.HCl$
Molecular weight: 360.91
CAS Registry No.: 130693-82-2
Merck Index: 3484
Lednicer No.: 5 149



SAMPLE

Matrix: blood

Sample preparation: Whole blood, plasma. 1 mL Whole blood or plasma + 600 μL 10% trichloroacetic acid, vortex for 1 min, add 7 mL 200 mM pH 8 phosphate buffer, vortex, add 10 mL toluene:ethyl acetate:isopropanol 40:50:10, shake on a flat-bed shaker for 20 min, centrifuge at 2060 g for 5 min. Remove 8 mL of the upper organic phase and add it to 300 μL 15 mM phosphoric acid, shake at 60 shakes/min for 20 min, centrifuge, inject a 200 μL aliquot of the aqueous layer. Urine. 1 mL Urine + 1 mL 200 mM pH 8 phosphate buffer, vortex, add 10 mL toluene:ethyl acetate:isopropanol 40:50:10, shake on a flat-bed shaker for 20 min, centrifuge at 2060 g for 5 min. Remove 8 mL of the upper organic phase and add it to 300 μL 15 mM phosphoric acid, shake at 60 shakes/min for 20 min, centrifuge, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 25 × 4.6 5 μm RP-18 (Brownlee)
Column: 250 × 4.6 5 μm RP-8 (Beckman) + 50 × 4.6 3 μm RP-18 (Analytichem) in series
Mobile phase: MeCN:buffer 25:75 (plasma) or 24:76 (urine) (Buffer was 0.085% phosphoric acid containing 1.6 g/L sodium octanesulfonate.)
Flow rate: 1
Injection volume: 200
Detector: UV 252

CHROMATOGRAM

Retention time: 17.5
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; plasma; urine

REFERENCE

Matuszewski,B.K.; Constanzer,M.L.; Woolf,E.J.; Au,T.; Haddix,H. Determination of MK-507, a novel topically effective carbonic anhydrase inhibitor, and its de-ethylated metabolite in human whole blood, plasma, and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *653*, 77–85.

SAMPLE

Matrix: blood, incubations

Sample preparation: Incubation mixtures. Centrifuge, filter (Ultrafree C3LGC), inject an aliquot of the ultrafiltrate. Whole blood. 1 mL Whole blood + 1 mL 6% trichloroacetic acid, mix, add 7 mL 200 mM pH 8.0 phosphate buffer, add 10 mL ethyl acetate, extract. Remove the organic layer and add it to 500 μL 25 mM phosphoric acid, extract, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere in series with 50 × 4.6 3 μm Bondesil (Analytichem)

Mobile phase: MeCN:25 mM KH_2PO_4 12:88
Column temperature: 40
Flow rate: 1
Detector: UV 252

KEY WORDS
whole blood; ultrafiltrate; incubations

REFERENCE
Hasegawa,T.; Hara,K.; Hata,S. Binding of dorzolamide and its metabolite, N-deethylated dorzolamide, to human erythrocytes in vitro, *Drug Metab.Dispos.*, **1994**, *22*, 377–382.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Whole blood + 600 μL 10% trichloroacetic acid, vortex for 1 min. Mix the acidified whole blood or 1 mL urine with 7 (blood) or 1 (urine) mL 200 mM pH 8.0 phosphate buffer, vortex for 10 s, add 10 mL toluene:ethyl acetate:isopropanol 49:50:1, shake mechanically at 60 strokes/min for 20 min, centrifuge for 5 min. Remove an 8 mL aliquot of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 300 μL 5 μL/mL (+)-(S)-1-(1-naphthyl)ethyl isocyanate in dry dichloromethane, vortex for 2 min, let stand at room temperature overnight. Evaporate to dryness under a stream of nitrogen and reconstitute the residue in 300 μL 0.085% phosphoric acid, vortex for 2 min, add 2 mL hexane, vortex for 5 min, centrifuge. Remove a 250 μL aliquot of the loner aqueous layer and add it to 100 μL MeCN:MeOH 20:80, vortex, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (Baker)
Mobile phase: MeCN:MeOH:0.085% phosphoric acid 8:34:58
Flow rate: 1
Injection volume: 200
Detector: UV 252

CHROMATOGRAM
Retention time: 58.2 (4S,6S), 55.4 (4R,6R), 65.2 (4S,6R), 74.4 (4R,6S)

KEY WORDS
derivatization; chiral; whole blood

REFERENCE
Matuszewski,B.K.; Constanzer,M.L. Indirect chiral separation and analyses in human biological fluids of the stereoisomers of a thienothiopyran-2-sulfonamide (TRUSOPT), a novel carbonic anhydrase inhibitor with two chiral centers in the molecule, *Chirality*, **1992**, *4*, 515–519.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 600 μg/mL solution in MeOH:water 20:80, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 80 × 3 3 μm CR-C8 (Perkin-Elmer)
Mobile phase: Gradient. A was 1 L water containing 1 mL triethylamine and 1 mL acetic acid, pH 4.5 ± 0.1. B was MeCN. A:B for 100:0 for 10 min, to 50:50 over 20 min.
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
rugged

REFERENCE
Dovletoglou,A.; Thomas,S.M.; Berwick,L.; Ellison,D.K.; Tway,P.C. Development of practical HPLC methods for analysis and quality assessment of the novel carbonic anhydrase inhibitor MK-0507 and the acetamidosulfonamide intermediate, *J.Liq.Chromatogr.*, **1995**, *18*, 2337–2352.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in 0.085% phosphoric acid.

HPLC VARIABLES
Column: 125 × 4.6 5 μm DyChrom/ChemcoPak C8 + 50 × 4.6 3 μm C18 (Analytichem)
Mobile phase: MeCN:0.085% phosphoric acid 8:92
Flow rate: 1.2
Injection volume: 200
Detector: UV 252

CHROMATOGRAM
Retention time: 12

KEY WORDS
the drug (4S,6S) was not separated from the 4R,6R diastereomer but it was separated from the 4S,6R and 4R,6S diastereomers.

REFERENCE
Matuszewski,B.K.; Constanzer,M.L. Indirect chiral separation and analyses in human biological fluids of the stereoisomers of a thienothiopyran-2-sulfonamide (TRUSOPT), a novel carbonic anhydrase inhibitor with two chiral centers in the molecule, *Chirality*, **1992**, *4*, 515–519.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm 110 Å (R)-3,5-dinitrobenzoyl glycine (DNBPG) (Regis)
Mobile phase: Hexane:dichloromethane:MeOH 50:45:5
Flow rate: 2
Injection volume: 10-100
Detector: UV 252

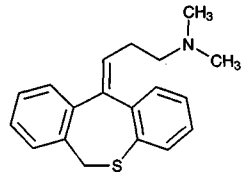
CHROMATOGRAM
Retention time: 7.1 (RR), 8.3 (RS), 9.2 (SS), 10.7 (SR)

KEY WORDS
chiral

REFERENCE

Matuszewski,B.K.; Constanzer,M.L.; Kiganda,M. Analytical chiral separation of the stereoisomers of a novel carbonic anhydrase inhibitor and its deethylated metabolite, and the assignment of absolute configuration of the human metabolite and chiral degradation products, *Pharm.Res.*, **1994**, *11*, 449–454.

Dothiepin



Molecular formula: $C_{19}H_{21}NS$
Molecular weight: 295.45
CAS Registry No.: 113-53-1, 897-15-4 (HCl)
Merck Index: 3485
Lednicer No.: 3 239

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 6.82
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: Blood, stomach contents. 1 mL Postmortem blood or stomach contents + 500 μL 1 M potassium carbonate + 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot. Urine. 1 mL Urine + 200 μL concentrated HCl, heat at 100° for 1 h, cool, adjust pH to 9.5-10 with KOH pellets and 1 M potassium carbonate, add 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot. Tissue. Add tissue to an equal volume isotonic saline, homogenize with an Ultra-Turraz mixer, remove a 2 g aliquot, add 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2.1 Chrompack pellicular reverse phase

Column: 100 × 3 5 μm Chromspher C8

Mobile phase: Gradient. MeOH:water containing 0.125% isopropylamine from 30:70 to 75:25 over 15 min

Flow rate: 0.7

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES

Simultaneous: trazodone, metabolites

REFERENCE

Lambert,W.; Van Bocxlaer,J.; Piette,M.; De Leenheer,A. A fatal case of trazodone and dothiepin poisoning: toxicological findings, *J.Anal.Toxicol.*, **1994**, *18*, 176–179.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:

1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 1υ
Detector: UV 254

CHROMATOGRAM

Retention time: 10.6 (A), 19.7 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, clomipramine, desipramine, doxepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.943

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Retention time: 8
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, doxepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergócornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 30 μL aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb S5SCX in a PEEK column
Mobile phase: MeOH:water:60% perchloric acid 97.5:1.75:0.75
Flow rate: 1
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

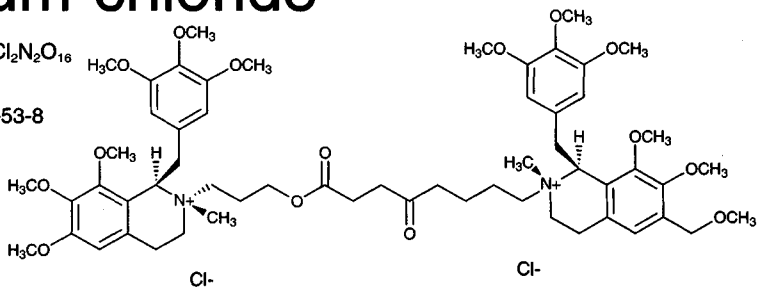
Simultaneous: clonazepam, diazepam, dothiepin sulfoxide, nordiazepam, nordothiepin sulfoxide, nordothiepin

REFERENCE

Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

Doxacurium chloride

Molecular formula: $C_{56}H_{78}Cl_2N_2O_{16}$
Molecular weight: 1106.14
CAS Registry No.: 106819-53-8
Merck Index: 3487



SAMPLE

Matrix: bile, blood, urine

Sample preparation: Condition a Bond Elut C1 SPE cartridge with 3 mL MeOH and 3 mL water, do not allow to dry. Dilute bile 1:3 with water. Add 50 μL 70 mg/mL phenylmethanesulfonyl fluoride (an enzyme inhibitor) in DMF to 5 mL blood before centrifuging at 3000 g for 10 min to prepare plasma. 1 mL Plasma, urine, or diluted bile + 200 ng mivacurium chloride in solvent, add to the SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, wash with 3 mL MeOH, wash with 3 mL water, elute with 1 mL eluant, mix eluate thoroughly, inject a 20-100 μL aliquot. (Solvent was MeOH:acidified saline 20:80. Acidified saline was 0.9% NaCl adjusted to pH 3 with 100 mM HCl. Eluant was MeOH:50 mM pH 3 KH_2PO_4 80:20.)

HPLC VARIABLES

Guard column: 5 μm Spherisorb S5C1 methylsilyl
Column: 150 × 4.6 5 μm Spherisorb S5C1 methylsilyl
Mobile phase: MeCN:MeOH:50 mM pH 3 KH_2PO_4 70:0.35:30
Flow rate: 1
Injection volume: 20-100
Detector: UV 210

CHROMATOGRAM

Retention time: 6.7
Internal standard: mivacurium chloride (5.7)
Limit of quantitation: 30 ng/mL (bile), 10 ng/mL (plasma, urine)

OTHER SUBSTANCES

Simultaneous: pancuronium, atracurium, tubocurarine succinylcholine, metocurine

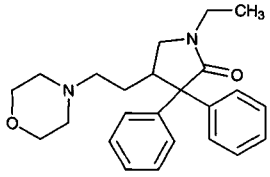
KEY WORDS

plasma; dog; human; SPE; pharmacokinetics

REFERENCE

DeAngelis,R.; Loebs,P.; Maehr,R.; Savarese,J.; Welch,R. High-performance liquid chromatographic analysis of doxacurium, a new long-acting neuromuscular blocker, *J.Chromatogr.*, **1990**, *525*, 389–400.

Doxapram



Molecular formula: $C_{24}H_{30}N_2O_2$
Molecular weight: 378.51
CAS Registry No.: 309-29-5, 7081-53-0 (HCl monohydrate), 113-07-5 (HCl)
Merck Index: 3488
Lednicer No.: 2 236

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 50 mM pH 11 borax buffer, vortex for 5 s, add 10 mL chloroform, shake for 10 min, centrifuge at 11400 g for 10 min, filter (Whatman No. 1 PS phase-separating paper). Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 μL mobile phase, inject the whole sample.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water:heptanesulfonic acid (Pic B7) 45:55:0.2 containing 2% glacial acetic acid
Flow rate: 2
Injection volume: 100
Detector: UV 225

CHROMATOGRAM

Retention time: 5.5
Internal standard: doxapram
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: xylazine

KEY WORDS

plasma; sheep; doxapram is IS

REFERENCE

Alvinerie,M.; Toutain,P.L. Determination of xylazine in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *222*, 308–310.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.

Column temperature: 30

Flow rate: 2

Injection volume: 5

Detector: UV 210

CHROMATOGRAM
Retention time: 14.0

OTHER SUBSTANCES
Simultaneous: acetaminophen, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: aprobarbital, vincamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylpredni-

solone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 9.10

KEY WORDS

narrow-bore; chiral; α 1.24

REFERENCE

Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 8.67 (A), 4.64 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

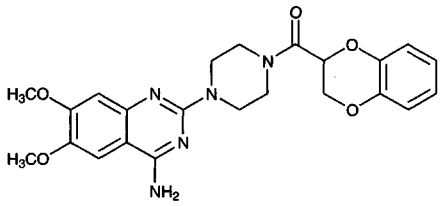
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Doxazosin



Molecular formula: $C_{23}H_{25}N_5O_5$
Molecular weight: 451.48
CAS Registry No.: 74191-85-8, 77883-43-3 (mesylate)
Merck Index: 3489
Lednicer No.: 4 148

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 M NaOH + IS, extract with 5 mL pentane: dichloromethane 2:1. Remove the organic layer and evaporate it to dryness under a gentle stream of nitrogen, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 10 μm Chiralpak AD (Chiral Technologies, Exton, PA)
Mobile phase: n-Hexane:isopropanol:diethylamine 70:30:0.1
Column temperature: 30
Flow rate: 0.2
Injection volume: 10
Detector: MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 452.0/344.0

CHROMATOGRAM

Retention time: 5.64 (R), 7.75 (S)
Internal standard: prazosin (4.50)

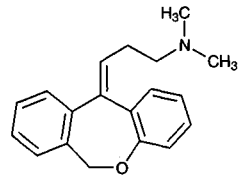
KEY WORDS

plasma; chiral; small-bore

REFERENCE

Alebic-Kolbah; T.; Zavitsanos; A. P. Chiral bioanalysis by normal high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *759*, 65–77.

Doxepin



Molecular formula: $C_{19}H_{21}NO$
Molecular weight: 279.38
CAS Registry No.: 1668-19-5, 1229-29-4 (HCl), 4698-39-9 (E), 25127-31-5 (Z), 3607-18-9 (trans HCl)
Merck Index: 3492
Lednicer No.: 1 404

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 2 mL 500 mM NaOH, vortex briefly, add 4 mL heptane:isoamyl alcohol 98.5:1.5 and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL heptane: isoamyl alcohol 98.5:1.5 to extraction sample, mix. Combine the organic layers and extract them with 2 mL 50 mM sulfuric acid. Make the acid layer alkaline with 1 mL 1.0 M pH 9.0 carbonate/bicarbonate buffer and mix with 2 mL toluene:isoamyl alcohol 85:15 for 15 min. Evaporate the organic layer to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II OD
Mobile phase: MeCN:pH 3 phosphate buffer:n-nonylamine 40-50:60:0.12
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.99
Internal standard: doxepin

OTHER SUBSTANCES

Extracted: amitriptyline, nortriptyline

KEY WORDS

liver; lung; muscle; urine; pericardial fluid; doxepin is IS

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 30 mg Oasis HLB SPE cartridge with 1 mL MeOH and 1 mL water. Acidify (?) mL serum with 20 μL phosphoric acid, vortex for 5 s, add to the SPE cartridge, wash with 1 mL MeOH :water 5:95, elute with 1 mL MeOH. Evaporate the eluate to dryness at 40° under a stream of nitrogen. Reconstitute the residue with 200 μL MeOH:20 mM pH 7 phosphate buffer 20:80, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 Sentry
Column: 150 × 3.9 5 μm Symmetry C18 (Waters)

Mobile phase: MeOH:20 mM pH 7 potassium phosphate 70:30
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Internal standard: nordoxepin (4.9)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, nortriptyline

KEY WORDS
pig; serum; SPE

REFERENCE
Cheng,Y.-F.; Phillips,D.J.; Neue,U.; Bean,L. Solid-phase extraction for the determination of tricyclic antidepressants in serum using a novel polymeric extraction sorbent, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2461–2473.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 μL 10 μg/mL protriptyline in water + 200 μL 80 g/L $NaHCO_3$ + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100 μL mobile phase, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3.03
Internal standard: protriptyline (12.20)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES
Simultaneous: imipramine, trimipramine, amitriptyline, desmethyldoxepin, nortriptyline, desipramine, chlorpromazine, thioridazine, propranolol, propoxyphene, disopyramide, maprotiline
Noninterfering: caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen, trifluoperazine
Interfering: procainamide

KEY WORDS
serum

REFERENCE
Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE
Matrix: blood

Sample preparation: Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then 200 μL 700 ng/mL promazine in MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200 μL 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200 μL mobile phase, vortex 10 s, inject 75-100 μL aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25 μL concentrated HCl.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM

Retention time: 3.6, 3.3 (cis isomer)
Internal standard: promazine (5.2)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, desmethyldoxepin, imipramine, nortriptyline, protriptyline
Simultaneous: N-acetylprocainamide, procainamide, zimeldine, morphine, codeine, trifluoperazine, desmethyldisopyramide, 10-hydroxynortriptyline, prochlorperazine, oxaprotiline, 2-hydroxydesipramine, chlorpheniramine, maprotiline, norzimeldine, iminostilbene, desmethylchlordiazepoxide, buprion, diazepam, demoxepam, chlordiazepoxide, propoxyphene, dextropropoxyphene, cocaine, oxapam, trimipramine, mianserin, trimeprazine, loxepin, fluphenazine, methadone, trifluopromazine, phenteramine, chlorimipramine, perphenazine, quinidine
Noninterfering: thiopropazine
Interfering: thioridazine, hydroxyamoxapine, meperidine, chlorpromazine, disopyramide, amphetamine, 2-hydroxyimipramine, iprindole, pyrilamine, promethazine, prolixin, amoxapine

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KEY WORDS

serum; normal phase

REFERENCE

Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane:isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2

Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: k' 4.18
Internal standard: loxapine (k' 7.18)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, fluphenazine, haloperidol, imipramine, nortriptyline, oxazepam, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: desmethyldiazepam

KEY WORDS

plasma

REFERENCE

Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES

Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: N-desmethylclomipramine hydrochloride (10)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: desmethyldoxepin, metabolites
Also analyzed: desmethylclomipramine, clomipramine, maprotiline, protriptyline, amitriptyline, nortriptyline, imipramine, 2-hydroxyimipramine, 2-hydroxydesipramine, desipramine
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

KEY WORDS

plasma

REFERENCE

Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 1 mL 450 mM NaOH + 5 mL hexane:isopropanol 95:5, shake for 5 min, centrifuge. Remove 4 mL of the organic layer and add it to 50 μL 200 mM HCl, shake for 2 min, centrifuge. Inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)

Mobile phase: MeCN:500 mM NaH_2PO_4 35:65 adjusted to pH 2.2 with phosphoric acid

Flow rate: 0.8

Injection volume: 20

Detector: UV 210

CHROMATOGRAM

Retention time: 3.3

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: nortriptyline, amitriptyline

KEY WORDS

serum

REFERENCE

Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Analytichem cyanopropyl SPE cartridge with 1 mL water and 1 mL MeOH, do not allow to dry. Add 1 mL serum + 250 μL 0.2 μg/mL imipramine in 50 mM sodium n-heptanesulfonic acid to the SPE cartridge, wash with 1 mL water, 1 mL MeOH:water 50:50, air dry cartridge, elute with 1 mL MeOH:triethylamine 99.2:0.8, evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute residue with 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5μm Spherisorb cyanopropyl

Mobile phase: MeOH:20 mM phosphoric acid containing 0.05% N,N-diethyloctylamine 55:45, pH was 2.4

Flow rate: 1.5

Injection volume: 50

Detector: UV 214

CHROMATOGRAM

Retention time: 6

Internal standard: imipramine (9)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

serum; SPE

REFERENCE

Emm,T.; Lesko,L.J.; Perkal,M.B. Simultaneous determination of doxepin and nordoxepin in serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *419*, 445–451.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 200 ng desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 12.2
Internal standard: desipramine (14.2)

OTHER SUBSTANCES

Extracted: cocaine, dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS

doxepin is also IS; serum

REFERENCE

Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μL Serum + 50 μL 1 μg/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200 μL 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 8.1
Internal standard: N-propionylprocainamide (6)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, imipramine, nortriptyline, protriptyline, trimipramine

Simultaneous: atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine

Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil

KEY WORDS

serum; SPE

REFERENCE

Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE

Matrix: blood

Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES

Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)

Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate

Flow rate: 1

Injection volume: 200

Detector: UV 210

CHROMATOGRAM

Retention time: 9.5

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, clomipramine, desipramine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS

serum; column-switching; use gradient to determine metabolites

REFERENCE

Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE

Matrix: blood

Sample preparation: For each 1 mL plasma or serum add 10 μL 14 μg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile

phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K_2HPO_4 adjusted to pH 6.8 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM

Retention time: 8.26
Internal standard: trimipramine (6.5)
Limit of detection: 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, desipramine, fluvoxamine, imipramine, maprotiline, nortriptyline
Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol
Interfering: amitriptyline, clomipramine

KEY WORDS

plasma; serum; column-switching

REFERENCE

Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

SAMPLE

Matrix: blood
Sample preparation: Add 10 μL 20 μg/mL oxaprotiline in MeOH to 990 μL plasma or serum. Inject 100 μL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM

Retention time: 8.3
Internal standard: oxaprotiline (9.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: clozapine, fluvoxamine, metoclopramide, fluoxetine, imipramine, norfluoxetine, nortriptyline, desipramine, maprotiline

Noninterfering: haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine
Interfering: amitriptyline, clomipramine

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Wetzel,H.; Hiemke,C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography, *Clin.Chem.*, **1992**, *38*, 2082–2086.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 3.2
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, encainide, fentanyl, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: diphenhydramine, flecainide, haloperidol, trazodone

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 990 μL Serum + 10 μL 14 μg/mL trimipramine in MeOH. Inject onto column A and elute with mobile phase A for 15 min then elute contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 10 μm Hypersil MOS C8; B 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:5778:235 (sic, perhaps 188:577:235 ?)
Detector: UV 214

CHROMATOGRAM
Internal standard: trimipramine
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; column-switching

REFERENCE
Rao,M.L.; Staberock,U.; Baumann,P.; Hiemke,C.; Deister,A.; Cuendet,C.; Amey,M.; Härtter,S.; Kraemer,M. Monitoring tricyclic antidepressant concentrations in serum by fluorescence polarization immunoassay compared with gas chromatography and HPLC, *Clin.Chem.*, **1994**, *40*, 929–933.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 75 μL MeCN containing 4 mg/mL amoxapine and 4 mg/mL loxapine + 2 mL 250 mM NaOH + 400 μL isoamyl alcohol, vortex vigorously, let stand for 5 min, add 10 mL heptane, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Remove the upper heptane layer and add it to 1 mL 100 mM pH 3 glycylglycine buffer, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Discard the heptane layer, add 1 mL 250 mM NaOH to the aqueous layer, add 5 mL n-pentane, shake for 1 h, centrifuge at 2000 g for 30 min. Remove the organic layer and evaporate it to dryness under educed pressure, reconstitute the residue in 70 μL mobile phase, vortex vigorously, centrifuge at 2000 g for 2-3 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 40 μm pellicular silica (CEL Associates, Houston)
Column: 100 × 6 3 μm silica 80 Å (CEL Associates, Houston)
Mobile phase: MeCN:buffer 20:80 containing 21 mM n-nonylamine, pH 7.4-7.8 (Buffer was 25 mM Na_2HPO_4 adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1.6
Injection volume: 50
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 14.70 (Z isomer), 16.40 (E isomer)

Internal standard: amoxapine (9.63), loxapine (22.68)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, chlorpromazine, clomipramine, desipramine, fluoxetine, imipramine, mianserin, nortriptyline, thioridazine, trimipramine
Noninterfering: diazepam

KEY WORDS
serum

REFERENCE
Adamczyk,M.; Fishpaugh,J.R.; Harrington,C. Quantitative determination of *E*- and *Z*-doxepin and *E*- and *Z*-desmethyldoxepin by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 371–376.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 200 μL Plasma or whole blood + 50 μL 100 μM testosterone propionate in MeOH + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 30:70, dry, elute with 200 μL 95% EtOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-8DB
Mobile phase: MeOH:buffer 72.5:27.5 (Buffer was 25 mM K_2HPO_4 adjusted to pH 3 with 670 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.75
Internal standard: testosterone propionate (3.60)

OTHER SUBSTANCES
Extracted: clotrimazole, itraconazole
Noninterfering: acetaminophen, N-acetylprocainamide, amitriptyline, aspirin, barbituric acid, brompheniramine, caffeine, carbamazepine, chloramphenicol, chlorpheniramine, clonazepam, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, ethosuximide, felbamate, gentamicin, ibuprofen, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, procainamide, protriptyline, quinidine, theophylline, tobramycin, trimethadione, valproic acid, vancomycin

KEY WORDS
plasma; SPE; whole blood

REFERENCE
Rifai,N.; Sakamoto,M.; Law,T.; Platt,O.; Mikati,M.; Armsby,C.C.; Brugnara,C. HPLC measurement, blood distribution, and pharmacokinetics of oral clotrimazole, potentially useful antisickling agent, *Clin.Chem.*, **1995**, *41*, 387–391.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 296

CHROMATOGRAM

Retention time: 6.31
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + NaOH + hexane:isoamyl alcohol 98:2, extract. Remove the organic phase and add it to 0.03% phosphoric acid, extract, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: C18
Column: 100 × 8 10 μm Resolve C8 (Waters)
Mobile phase: MeCN:MeOH:56 mM ammonium acetate:1 M ammonium hydroxide 100:10:4.5:2.6
Flow rate: 2.5
Detector: UV 220

CHROMATOGRAM
Retention time: 11.6
Internal standard: doxepin

OTHER SUBSTANCES
Extracted: norfluoxetine, fluoxetine, amitriptyline, nortriptyline

KEY WORDS
plasma; doxepin is IS

REFERENCE
el-Yazigi,A.; Chaleby,K.; Gad,A.; Raines,D.A. Steady-state kinetics of fluoxetine and amitriptyline in patients treated with a combination of these drugs as compared with those treated with amitriptyline alone, *J.Clin.Pharmacol.*, **1995**, *35*, 17–21.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 10.5
Internal standard: cianopramine (8.93)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: brompheniramine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)

Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40

Flow rate: 0.6

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 8.5 (A), 10.3 (B)

Internal standard: diazepam (4.4, A)

Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 500 μL 3 M ammonia solution and 7 mL n-pentane:isopropanol 95:5 to 2 mL plasma containing 200 ng/mL nortriptyline or 2 mL urine containing 400 ng/mL nortriptyline, shake in an overhead shaker for 20 min, let stand for 10 min. Transfer the upper organic layer to a tube containing 1 mL 100 mM HCl, shake for 20 min, let stand for 5 min. Aspirate the organic phase to waste, wash the remaining aqueous layer with 3 mL pentane by shaking for 10 min. Add 500 μL 3 M ammonia solution and 6 mL a-pentane:isopropanol 95:5 to the washed aqueous layer, shake for 20 min, evaporate the organic layer under a stream of nitrogen at 65°, reconstitute the residue with 160 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb silica

Mobile phase: MeOH:hexane:nonylamine 5:95:0.3

Flow rate: 1

Injection volume: 60

Detector: UV 254

CHROMATOGRAM

Retention time: 4.0 (cis), 4.5 (trans)

Internal standard: nortriptyline (10)

OTHER SUBSTANCES

Extracted: metabolites, N-desmethyldoxepin

KEY WORDS

dog; human; plasma; normal phase

REFERENCE

Yan,J.; Hubbard,J.W.; McKay,G.; Midha,K.K. Stereoselective and simultaneous measurement of cis- and trans-isomers of doxepin and N-desmethyldoxepin in plasma or urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *691*, 131–138.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 14.095

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, dothiepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2

Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)

Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: amitriptyline, desmethyldoxepin, desipramine, imipramine, loxapine, maprotiline, nortriptyline, trazodone

REFERENCE
Supelco Catalog, **1993**, p. 440.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 6.4
Limit of quantitation: < 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: desipramine, protriptyline, cyclobenzaprine, maprotiline
Also analyzed: amitriptyline, amoxapine, carbamazepine, imipramine, nortriptyline

KEY WORDS
UV spectra given

REFERENCE
Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: desmethyldoxepin, desipramine, nortriptyline, imipramine, amitriptyline

Also analyzed: amphetamine, chlordiazepoxide, chlorpromazine, desalkylflurazepam, diazepam, diethylpropion, ephedrine, fenfluramine, flurazepam, mesoridazine, methamphetamine, norchlordiazepoxide, nordiazepam, oxazepam, phentermine, phenylpropanolamine, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7, mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recin-

namine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 12.90 (A), 6.06 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyra-

zone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: vitreous humor
Sample preparation: 600 μL Vitreous humor + 3 mL 0.1 M NaCl + 50 μL 4 μg/mL desmethylclomipramine in water, mix for a few s, add to a C18 SepPak attached to a 5 mL syringe, allow to flow through (10-15 min). Wash with 1 mL 0.1 M NaCl, wash with 1 mL water, wash 3 mL reagent by gravity. Elute with 3 mL MeOH and push air through to remove as much as possible. Evaporate in vacuum at 37°, vortex with 50 μL mobile phase for 1 min, inject 25 μL aliquot. (Reagent was isopropanol:n-heptane:1 M sulfuric acid 40:320:1.)

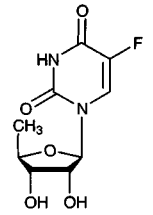
HPLC VARIABLES
Guard column: 50 × 4.6 30 μm Permaphase ETH
Column: 250 × 4.6 5-6 μm Zorbax cyanopropyl
Mobile phase: MeCN:0.5 M acetic acid:n-butylamine 40:60:0.0022
Flow rate: 2.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 19.5
Internal standard: Desmethylclomipramine
Limit of detection: 16.7 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, nortriptyline, imipramine, metabolites
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, diazepam, digoxin, disopyramide, ethosuximide, flurazepam, gentamicin, haloperidol, kanamycin, lidocaine, meprobamate, methapyriline, methaqualone, methotrexate, methyprylon, netilmicin, pentazocine, pentobarbital, phenobarbital, phenytoin, prazepam, primidone, procainamide, propranolol, quinidine, salicylic acid, secobarbital, streptomycin, theophylline, tobramycin, tocainide, valproic acid, vancomycin.

REFERENCE
Evenson,M.A.; Engstrand,D.A. A SepPak HPLC method for tricyclic antidepressant drugs in human vitreous humor, *J.Anal.Toxicol.*, **1989**, *13*, 322–325.

Doxifluridine



Molecular formula: $C_9H_{11}FN_2O_5$
Molecular weight: 246.20
CAS Registry No.: 3094-09-5
Merck Index: 3493

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL LC-SCX Supelclean strong cation-exchange SPE cartridge (Supelco) with 2 mL MeOH, 1 mL 100 mM copper(II) sulfate solution, and 3 mL 50 mM pH 7 phosphate buffer, do not allow to dry. 300 µL Serum + 5-bromouracil, add to the SPE cartridge, wash with 2 mL 50 mM pH 7 phosphate buffer, wash with 2 mL MeOH, elute with 700 µL 1.7 M ammonia solution, add 70 µL glacial acetic acid to the eluate, mix thoroughly, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 µm Supelguard LC-18-S (Supelco)
Column: 250 × 4.6 5 µm Supelcosil LC-18-S ODS
Mobile phase: Gradient. A was MeOH:50 mM pH 6.5 phosphate buffer 60:40. B was 50 mM pH 6.5 phosphate buffer.
Flow rate: 1
Injection volume: 20
Detector: UV 269

CHROMATOGRAM

Retention time: 15.5
Internal standard: 5-bromouracil (12)
Limit of detection: 60 ng/mL

OTHER SUBSTANCES

Extracted: 5-fluorouracil, floxuridine, 5-fluorouridine monophosphate, metabolites

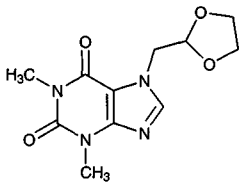
KEY WORDS

serum; SPE; pharmacokinetics

REFERENCE

Guerrieri,A.; Palmisano,F.; Zambonin,P.G.; De Lena,M.; Lorusso,V. Solid-phase extraction of fluoropyrimidine derivatives on a copper-modified strong cation exchanger: determination of doxifluridine, 5-fluorouracil and its main metabolites in serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 71–77.

Doxofylline



Molecular formula: $C_{11}H_{14}N_4O_4$
Molecular weight: 266.26
CAS Registry No.: 69975-86-6
Merck Index: 3494
Lednicer No.: 5 144

SAMPLE

Matrix: blood
Sample preparation: Centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm internal surface reversed phase Pinkerton, silica derivatized with glycine-phenylalanine-phenylalanine (Regis) (periodically reverse the column)
Mobile phase: 100 mM pH 6.8 phosphate buffer
Flow rate: 0.3
Injection volume: 10
Detector: UV 275

CHROMATOGRAM

Retention time: 13.94
Limit of detection: <1000 ng/mL

OTHER SUBSTANCES

Extracted: dyphylline, theophylline, caffeine
Noninterfering: acetaminophen, amitriptyline, amphetamine, atropine, benzoylecgonine, benztropine, caffeine, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, chlorprothixene, cimetidine, cocaine, codeine, dextromethorphan, diazepam, diphenhydramine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, flurazepam, glutethimide, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, nicotine, nordiazepam, nortriptyline, orphenadrine, papaverine, pentazocine, phenacetin, phencyclidine, phenmetrazine, phenolphthalein, phentermine, phenylpropanolamine, phenytoin, prazepam, procainamide, procaine, propoxyphene, propranolol, protriptyline, pseudoephedrine, pyrilamine, quinine, salicylamide, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, trimethobenzamide, trimethoprim, tripelennamine

KEY WORDS

plasma; serum; direct injection

REFERENCE

Tagliaro,F.; Dorizzi,R.; Frigerio,A.; Marigo,M. Non-extraction HPLC method for simultaneous measurement of dyphylline and doxofylline in serum, *Clin.Chem.*, **1990**, *36*, 113–115.

SAMPLE

Matrix: blood, formulations, tissue
Sample preparation: Elixir. Make up 1 mL elixir to 50 mL with mobile phase. Remove a 5 mL aliquot and add it to 2 mL 500 μg/mL theophylline in mobile phase, make up to 100 mL with mobile phase, inject a 20 μL aliquot. Tablets. Finely powder a tablet in a mortar, weigh out 310 mg and add it to 100 mL mobile phase, let stand for some time, filter. Add 1 mL filtrate to 2 mL 500 μg/mL theophylline in mobile phase, make up to 100 mL with mobile phase, inject a 20 μL aliquot. Tissue. Homogenize rat brain, extract on

an Extrelut 3 SPE cartridge at pH 8, elute with chloroform, take up the residue in MeOH, inject an aliquot (from Drug Metab.Dispos. 1989, 17, 437). Serum. Extract on an Extrelut 3 SPE cartridge at neutral pH, elute with chloroform, take up the residue in MeOH, inject an aliquot (from Drug Metab.Dispos. 1989, 17, 437).

HPLC VARIABLES

Column: 250 mm long 10 μm LiChrosorb RP-18
Mobile phase: MeCN:water 30:70
Flow rate: 1.5
Injection volume: 20
Detector: UV 273

CHROMATOGRAM

Retention time: 2.6
Internal standard: theophylline (2)

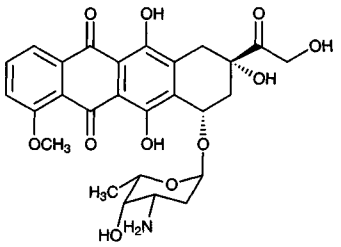
KEY WORDS

serum; rat; brain; elixir; tablets; SPE

REFERENCE

Badini,C.; Masera,F.; Franzone,J.S. Dosaggio del 2-(7'-teofillinmetil)-1,3-diossolano mediante HPLC [Assay of 2-(7'-theophyllinmethyl)-1,3-dioxolane using HPLC], *Farmaco.[Prat].*, **1982**, *37*, 320–324.

Doxorubicin



Molecular formula: $C_{27}H_{29}NO_{11}$
Molecular weight: 543.53
CAS Registry No.: 23214-92-8, 25316-40-9 (HCl)
Merck Index: 3495

SAMPLE

Matrix: bile, blood, feces, tissue, urine
Sample preparation: Homogenize tissue in 4% BSA in water to a final concentration of 0.05-2 g/mL. Homogenize feces in 4% BSA in water to a final concentration of 0.03-1 g/mL. Dilute feces homogenate 20-fold, urine 100-fold, and bile 20-fold with blank human plasma. 200 μL Sample + 200 μL 6% (w/v) pH 9.5 borate buffer + 100 μL 4 mg/mL IS in pH 2.05 water, vortex. Mix with 1 mL chloroform:1-propanol 20:80 for 5 min. (Caution! Chloroform is a carcinogen!) Centrifuge at 3000 g at 4° for 10 min. Remove the organic layer and evaporate it under reduced pressure at 43°. Reconstitute the residue in 100 μL MeCN:THF 40:1, vortex for 20 s, sonicate for 5 min. Add 300 μL water acidified to pH 2.05, vortex, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 pellicular RP material
Column: 100 × 3 7μm Lichrosorb RP-8
Mobile phase: MeCN:THF:water adjusted to pH 2.05 with perchloric acid 30:1:80
Flow rate: 0.4
Injection volume: 50
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 6
Internal standard: daunorubicin (12)
Limit of quantitation: 1.8-2.4 nM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

mouse; plasma; brain; muscle; colon; cecum; small intestine; stomach; liver; gall bladder; kidney; lung; spleen; heart; ovary; uterus; breast; testis; epididymis; eye; pharmacokinetics

REFERENCE

van Asperen,J.; van Tellingen,O.; Beijnen,J.H. Determination of doxorubicin and metabolites in murine specimens by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *712*, 129–143.

SAMPLE

Matrix: blood
Sample preparation: Condition an Oasis HLB SPE cartridge (Waters) with 1 mL mobile phase:water 1:3. 200 μL Plasma + 500 mL mobile phase:water 1:3 + 100 μL water, vortex, add to SPE cartridge, wash with 1 mL mobile phase:water 1:3, elute with 600 μL MeCN:mobile phase 1:1, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 200 × 4.6 10 μm LiChrosorb RP18

Mobile phase: MeCN:water 29:71 containing 50 mM Na_2HPO_4 and 0.05% (v/v) triethylamine adjusted to pH 4.6 with citric acid
Flow rate: 1.0
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5014 high-performance analytical cell containing amperometric electrode +400 mV coupled with coulometric electrode -300 mV, palladium reference electrode

CHROMATOGRAM
Retention time: 12.0
Internal standard: epirubicin (9.4)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; Fattorossi,A.; Zuccaro,R. Simultaneous determination of epirubicin, doxorubicin and their principal metabolites in human plasma by high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.B*, **1998**, *707*, 219–225.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 5 mL chloroform:isopropanol 80:20, extract, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30° in the dark, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 Nucleosil 100-5 C18
Mobile phase: MeCN:10 mM pH 4 ammonium formate buffer 30:70
Flow rate: 1.5
Injection volume: 25
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 4.5
Internal standard: doxorubicin

OTHER SUBSTANCES
Extracted: epirubicin

KEY WORDS
plasma; doxorubicin is IS

REFERENCE
Jakobsen,P.; Steiness,E.; Bastholt,L.; Dalmark,M.; Lorenzen,A.; Petersen,D.; Gjedde,S.B.; Sandberg,E.; Rose,C.; Nielsen,O.S. Multiple-dose pharmacokinetics of epirubicin at four different dose levels: studies in patients with metastatic breast cancer, *Cancer Chemother.Pharmacol.*, **1991**, *28*, 63–68.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or blood + 3 mL 100 mM pH 9.5 ammonia-ammonium chloride buffer + 20 ng daunorubicin + 13.5 mL chloroform:MeOH 2:1, shake mechanically

for 30 min, centrifuge at 3000 g for 10 min, repeat the extraction with 9 mL chloroform. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 3 mL chloroform:MeOH 2:1, evaporate this mixture, reconstitute the residue in 300 μL mobile phase, centrifuge a 75 μL aliquot at 10000 g for 1 min, inject the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm STR ODS-M (Shimadzu)
Mobile phase: MeCN:buffer 30:70 (Buffer was 200 mM acetic acid-ammonium formate, pH 4.0.)
Column temperature: 22
Flow rate: 0.7
Injection volume: 75
Detector: F ex 470 em 550

CHROMATOGRAM
Retention time: 7.7
Internal standard: daunorubicin (16.2)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: pirarubicin

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Nagasawa,K.; Yokoyama,T.; Ohnishi,N.; Iwakawa,S.; Okumura,K.; Kosaka,Y.; Sano,K.; Murakami,R.; Nakamura,H. Pharmacokinetics of pirarubicin in pediatric patients, *J.Pharmacobiodyn.*, **1991**, *14*, 222–230.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL 100 ng/mL daunorubicin in mobile phase, extract with 3 mL MeCN for 10 min, add 100 mg NaCl, shake for 5 min, centrifuge at 995 g for 15 min, let stand at -20° for 1 h. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Spherisorb phenyl
Column: 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:30 mM citrate buffer adjusted to pH 4 with formic acid 30:70
Column temperature: 50
Flow rate: 1.5
Injection volume: 100
Detector: F ex 480 em 590

CHROMATOGRAM
Retention time: 6.45
Internal standard: daunorubicin (8.5)
Limit of detection: 0.5 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: pirarubicin, doxorubicinol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jacquet,J.M.; Galtier,M.; Bressolle,F.; Jourdan,J. A sensitive and reproducible HPLC assay for doxorubicin and pirarubicin, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 343–348.

SAMPLE
Matrix: blood, cells
Sample preparation: Thaw cell samples, sonicate (Branson B-12) at 50 W for 20 s. 400 μL Cell sample or plasma + 200 μL 200 nM daunorubicin in 100 mM pH 9.3 borate buffer, add 1.8 mL chloroform:MeOH 80:20, extract, inject a 200-500 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 250 × 4 Lichrosorb Si-60
Mobile phase: Chloroform:MeOH:glacial acetic acid:0.3 mM magnesium chloride 72:21:2:3
Flow rate: 1.5
Injection volume: 200-500
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 4.3
Internal standard: daunorubicin (3.3)
Limit of detection: 0.5 nM

OTHER SUBSTANCES
Extracted: metabolites, epirubicin

KEY WORDS
plasma; pharmacokinetics; normal phase

REFERENCE
Tidefelt,U.; Sundman-Engberg,B.; Paul,C. Comparison of the intracellular pharmacokinetics of doxorubicin and 4'-epi-doxorubicin in patients with acute leukemia, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 225–229.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix plasma with four volumes daunorubicin aglycone in MeOH, centrifuge, inject a 100 μL aliquot of the supernatant. Tissue. Tissue + 1 volume MeOH + 2 volumes 1 M pH 8.5 Tris buffer, homogenize, let stand on ice for 15 min, add 7 volumes MeCN containing daunorubicin aglycone, vortex, let stand at room temperature for 15 min, centrifuge, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 10 μm LiChrosorb RP-18
Column: 250 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:buffer from 15:85 to 50:50 over 10 min, re-equilibrate at initial conditions for 15 min. (Buffer was 25 mM $(NH_4)H_2PO_4$ and 30 mM phosphoric acid.)
Flow rate: 1.5
Injection volume: 100
Detector: F ex 475 em 580

CHROMATOGRAM
Retention time: 9
Internal standard: daunorubicin aglycone (13.5)

Limit of quantitation: 1.5 pmole

OTHER SUBSTANCES
Extracted: metabolites, 13-dihydrodoxorubicin

KEY WORDS
plasma; mouse; lung

REFERENCE
Rose,L.M.; Tillery,K.F.; el Dareer,S.M.; Hill,D.L. High-performance liquid chromatographic determination of doxorubicin and its metabolites in plasma and tissue, *J.Chromatogr.*, **1988**, *425*, 419–423.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. Serum + daunorubicin + 8 mL chloroform:isopropanol 50:50, extract, centrifuge at 3000 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot. Tissue. Homogenize tissue in water, add daunorubicin, add 30 μL silver nitrate (33%), extract with 8 mL isopropanol. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 300 mm long 10 μm μBondapak C18
Mobile phase: MeCN:water:100 mM phosphoric acid 37:37:26
Flow rate: 1.5
Injection volume: 5-20
Detector: F ex 475 em 580

CHROMATOGRAM
Internal standard: daunorubicin
Limit of detection: 20 ng/g (tissue), 5 ng/mL (serum)

KEY WORDS
serum; rat; pharmacokinetics; heart; liver; kidney; adrenal; brain; intestine; mouse

REFERENCE
Colombo,T.; Zucchetti,M.; D'Incalci,M. Cyclosporin A markedly changes the distribution of doxorubicin in mice and rats, *J.Pharmacol.Exp.Ther.*, **1994**, *269*, 22–27.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 150 μL Plasma + 150 μL MeCN, vortex for 10 s. Centrifuge at 3000 rpm for 5 min. Remove 200 μL of the organic layer, evaporate under reduced pressure, reconstitute the residue in 100 μL 100 mM pH 3 monobasic phosphate buffer. Inject a 10 μL aliquot. Urine. Directly inject a 10 μL aliquot of urine. (Silanize glassware with 3% dichlorodimethylsilane in toluene, rinse with MeOH before use.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS/TM silica (Tosoh Co., Japan)
Mobile phase: MeCN:buffer 35:65 (Buffer was 100 mM monobasic phosphate (sic) containing 0.3% heptafluorobutyric acid, adjusted to pH 3 with NaOH. At the end of the analysis, wash the column with MeOH and MeOH:water 50:50.)
Flow rate: 1
Injection volume: 10
Detector: F ex 460 em 555

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 25 nM
Limit of quantitation: 2.5 μM

OTHER SUBSTANCES
Extracted: metabolites, daunorubicin

KEY WORDS
plasma

REFERENCE
Emara,S.; Morita,I.; Tamura,K.; Razee,S.; Masujima,T.; Mohamed,H.A.; El Gizawy,S.M.; El Rabbat,N.A. Utility of ion-pair chromatography for analysis of some anthracyclines in plasma and urine, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 681–692.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 3 mL 10 mM pH 9.0 ammonium chloride buffer, adjust pH to 9.0 with NaOH, add 6 mL chloroform:MeOH 2:1, shake for 5 min, centrifuge at 12000 g at 4° for 10 min, remove the organic layer, re-adjust the pH of the aqueous layer, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Nova-Pak C18 phenyl
Mobile phase: MeCN:35 mM pH 3.0 ammonium formate buffer 35:65
Flow rate: 0.8
Detector: F ex 254 em 550

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: pirarubicin, metabolites

KEY WORDS
plasma; SPE

REFERENCE
Raber,M.N.; Newman,R.A.; Lu,K.; Legha,S.; Gorski,C.; Benjamin,R.S.; Krakoff,I.H. Phase I clinical trial and pharmacokinetic evaluation of 4'-O-tetrahydropyranyladriamycin (THP-adriamycin), *Cancer Chemother.Pharmacol.*, **1989**, *23*, 311–315.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + daunorubicin + daunorubicinone + 5 mL chloroform:isopropanol 75:25, shake mechanically for 30 min, centrifuge at 4° at 1200 g for 15 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at room temperature. Add the aqueous phase to 2 mL 50 (plasma) or 500 (urine) mM pH 8.4 borate buffer, add 5 mL chloroform:isopropanol 75:25, shake mechanically for 30 min, centrifuge at 4° at 1200 g for 15 min. Remove the lower organic layer and add it to the residue from the first extraction, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 600 μL MeOH:500 mM phosphoric acid 50:50, vortex for 30 s, add 2 mL N-hexane, vortex for 30 s, centrifuge, inject an aliquot of the aqueous phase (omit the hexane wash for urine samples).

HPLC VARIABLES
Guard column: 30-38 μm pellicular ODS (Whatman)
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:10 mM pH 1.4 phosphate buffer 25:10:65
Flow rate: 0.58
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 9
Internal standard: daunorubicin (25), daunorubicinone (35)
Limit of quantitation: 12.2 ng/mL (urine), 0.31 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Fraier,D.; Frigerio,E.; Pianezzola,E.; Strolin Benedetti,M.; Cassidy,J.; Vasey,P. A sensitive procedure for the quantitation of free and N-(2-hydroxypropyl)methacrylamide polymer-bound doxorubicin (PK1) and some of its metabolites, 13-dihydrodoxorubicin, 13-dihydrooxorubicinone and doxorubicinone, in human plasma and urine by reversed-phase HPLC with fluorimetric detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 625–633.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 232.2

CHROMATOGRAM
Retention time: 12.057

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in water and dilute it with mobile phase to obtain a 50 μg/mL solution. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18 (Shandon HPLC, UK)
Mobile phase: The ratio MeOH:aqueous phase was modified. Aqueous phase was pH 2.2 citrate buffer, Tris buffer, pH 5.1 acetate buffer or pH 5.4 phosphate buffer. Counter ion was 2.54 mM triethylamine, 2-cyano-2-butylhexanoic acid or heptane sulfonic acid. (Details for preparation mobile phase in paper.)
Column temperature: 20 ± 0.3
Flow rate: 1.0
Injection volume: 50
Detector: UV 494

CHROMATOGRAM
Retention time: not specified

KEY WORDS
ion-pair chromatography

REFERENCE
Pepin,X.; Attali,L.; Domrault,C.; Gallet,S.; Metreau,J.M.; Reault,Y.; Cardot,P.J.P.; Imalalen,M.; Dubernet,C.; Soma,E.; Couvreur,P. On the use of ion-pair chromatography to elucidate doxorubicin release mechanism from polyalkylcyanoacrylate nanoparticles at the cellular level, *J.Chromatogr.B*, **1997**, *702*, 181–191.

SAMPLE
Matrix: bulk
Sample preparation: Free doxorubicin. Dissolve the sample in water, inject an aliquot. Bound doxorubicin. Mix 500 μL of an aqueous solution with 500 μL 2 M HCl, heat at 50° for 1.5 h, cool to room temperature, inject an aliquot within 2 h.

HPLC VARIABLES
Column: 250 × 4.6 Vydac C18
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was 0.1% trifluoroacetic acid. A:B from 25:75 to 30:70 in 10 min, to 80:20 in 5 min, maintain at 80:20 for 15 min.
Detector: UV

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 500 ng/mL
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: adriamycinone

REFERENCE
Configliacchi,E.; Razzano,G.; Rizzo,V.; Vigevani,A. HPLC methods for the determination of bound and free doxorubicin, and of bound and free galactosamine, in methacrylamide polymer-drug conjugates, *J.Pharm.Biomed.Anal.*, **1996**, *15*, 123–129.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL aliquot to 50 mL with 0.9% sodium chloride, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 100-5CN
Mobile phase: MeCN:MeOH:20 mM pH 4.5 ammonium dihydrogen phosphate in water 20:20:60, containing 10 mM sodium heptanesulfonate
Flow rate: 1.0
Injection volume: 25
Detector: UV 297

CHROMATOGRAM
Retention time: 4.1

OTHER SUBSTANCES
Simultaneous: degradation products, methylparaben, propylparaben, vincristine

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Nyhammar,E.K.; Johansson,S.G.; Seiving,B.E. Stability of doxorubicin hydrochloride and vincristine sulfate in two portable infusion-pump reservoirs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1171–1173.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 220 × 4.6 5 μm silica (Brownlee)
Mobile phase: MeCN:6.25 mM NaH_2PO_4 adjusted to pH 3.0 with concentrated phosphoric acid 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 216

CHROMATOGRAM
Retention time: 7.7
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: dacarbazine, ondansetron
Noninterfering: degradation products

KEY WORDS
injections; 5% dextrose

REFERENCE
King,D.T.; Stewart,J.T. HPLC determination of dacarbazine, doxorubicin, and ondansetron mixture in 5% dextrose injection on underivatized silica with an aqueous-organic mobile phase, *J.Liq.Chromatogr.*, **1993**, *16*, 2309–2323.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH_2PO_4 adjusted to pH 5.4 with 1 M NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: methyl paraben, ondansetron, vincristine, degradation products

KEY WORDS
injections; saline

REFERENCE
King,D.T.; Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of a vincristine, doxorubicin, and ondansetron mixture in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1994**, *17*, 1399–1411.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 6.48

OTHER SUBSTANCES
Simultaneous: granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 20:80 (A) or MeCN:30 mM sodium dihydrogen phosphate 23:77 (B)
Flow rate: 1
Injection volume: 20
Detector: UV 307 (A), UV 195 (B)

CHROMATOGRAM
Retention time: 11.2 (A), 5.4 (B)

OTHER SUBSTANCES
Simultaneous: granisetron (A), cyclophosphamide (B)

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm IB-SIL C8 (Phenomenex)
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM
Retention time: 7.3

OTHER SUBSTANCES
Simultaneous: morphine

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 5.9
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: atropine, codeine, dansylamide, dansylcadaverine, methylatropine, naphazoline, noscapine, xylometazoline

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: tissue

Sample preparation: Flush femurs with MeCN:water 50:50. Homogenize 3 spleens with 5 mL MeCN:water 50:50. Centrifuge extracts immediately at 1500 rpm for 5 min, inject an aliquot.

HPLC VARIABLES

Column: 300 × 16 Nucleosil C18 Bondasorb
Mobile phase: MeOH: 10 mM sodium acetate:acetic acid 65:35:1.3
Flow rate: 1
Detector: F ex 470 em 550

CHROMATOGRAM

Retention time: 9

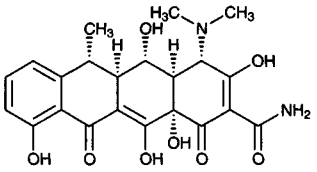
KEY WORDS

mouse; bone marrow; spleen

REFERENCE

Gibaud,S.; Demoy,M.; Andreux,J.P.; Weingarten,C.; Gouritin,B.; Couvreur,P. Cells involved in the capture of nanoparticles in hematopoietic organs, *J.Pharm.Sci.*, **1996**, *85*, 944–950.

Doxycycline



Molecular formula: $C_{22}H_{24}N_2O_8$
Molecular weight: 444.44
CAS Registry No.: 564-25-0, 17086-28-1 (monohydrate), 24390-14-5 (HCl monohydrate), 83038-87- 3 (fosfatex), 24390-14-5 (hyclate)
Merck Index: 3496

SAMPLE

Matrix: blood
Sample preparation: Add 100 μL MeCN:phosphoric acid:water 20:2:78 to 100 μL plasma, vortex for 10 s, centrifuge at 14000 g for 30 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 12.5 × 4 Zorbax RX-C18
Column: 150 × 4.6 5 μm Zorbax SB-C8
Mobile phase: MeCN:MeOH:water 14:10:76 containing 100 mM oxalic acid
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 350

CHROMATOGRAM

Retention time: 6-6.5
Limit of quantitation: 500 ng/mL

KEY WORDS

cat; plasma

REFERENCE

Kordick,D.L.; Papich,M.G.; Breitschwerdt,E.B. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2448–2455.

SAMPLE

Matrix: blood
Sample preparation: Add 50 μL 5 μg/mL demeclocycline in 1 mM HCl to 250 μL plasma, add 50 μL 6% aqueous ascorbic acid solution, 1 mL phosphate-sulfite buffer, and 6 mL ethyl acetate, vortex for 2 min, centrifuge at 2600 g for 10 min. Transfer 5 mL of the organic phase to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under nitrogen, redissolve the residue in 300 μL mobile phase, add 4 mL n-hexane, vortex, centrifuge at 2600 g for 5 min, remove the hexane layer by aspiration, inject a 100 μL aliquot of the aqueous phase. (The phosphate-sulfite buffer (pH 6) was 25.2 g sodium sulfite and 36.3 g NaH_2PO_4 dihydrate in 100 mL .)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher RP-18
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeCN:70% perchloric acid:water 29.85:0.25:69.9 containing 0.6 mM disodium EDTA and 5 mM oxalic acid, pH adjusted to 2.5 with 1 M NaOH
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 7.15

Internal standard: demeclocycline (4.28)
Limit of detection: 100 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
plasma; turkey

REFERENCE
Santos,M.D.; Vermeersch,H.; Remon,J.P.; Schelkens,M.; De Backer,P.; Ducatelle,R.; Haesebrouck,F. Validation of a high-performance liquid chromatographic method for the determination of doxycycline in turkey plasma, *J.Chromatogr.B*, **1996**, *682*, 301–308.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Prepare a metal chelate affinity chromatography (MCAC) column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH: water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Condition an SBD-RPS extraction membrane (3M Company, St. Paul, MN) with 2 mL MeOH and 2 mL 100 mM HCl. Add 20 mL 100 mM pH 4.0 sodium succinate buffer to 3 g pig kidney, pig muscle, cow liver, or whole chicken egg, vortex for 1 min and shake for 10 min on a horizontal shaker. Add 20 mL MeOH, sonicate for 5 min and centrifuge at 2666 g for 10 min at 4°. Filter the supernatant through a Whatman 541 filter paper. Add the clear supernatant to the MCAC column. Wash sequentially with 2 mL 100 mM sodium succinate buffer, 2 mL water, 2 mL MeOH, 2 mL water, and with 500 μL McIlvaine-EDTA-NaCl buffer. Elute with 3 mL McIlvaine-EDTA-NaCl buffer and adjust the eluate to pH 1.3 with 400 μL 4 M HCl. Add the eluate directly to the extraction membrane to prevent crystallization of EDTA. Wash the membrane with 1 mL 100 mM HCl and elute with four 250 μL portions of MeOH:25% ammonia 97:3, evaporate the eluate to dryness under the nitrogen at 40°. Reconstitute the dry residue with 250 μL 10 mM oxalic acid in water, vortex, sonicate. Inject a 100 μL aliquot. (The sodium succinate buffer was 100 mM succinic acid, pH adjusted to 4.0 with 10 M NaOH. Prepare the McIlvaine buffer by dissolving 12.9 g citric acid monohydrate and 10.9 g Na_2HPO_4 in 1 L water. The McIlvaine-EDTA-NaCl buffer was 100 mM EDTA and 500 mM NaCl in McIlvaine buffer. Protect all solutions from light.)

HPLC VARIABLES
Guard column: 5 × 3.0 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 8 μ PLRP-S (Polymer Laboratories)
Mobile phase: Gradient. A was 10 mM oxalic acid in water adjusted to pH 2.0 with 4 M HCl. B was MeCN. A:B from 85:15 to 60:40 over 16 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water stored at 4°.)

CHROMATOGRAM
Retention time: 20
Limit of detection: 3.00 ng/g (pig kidney), 1.38 ng/g (pig muscle), 2.35 ng/g (cow liver), 0.80 ng/g chicken egg)
Limit of quantitation: 5 ng/g (pig kidney)

OTHER SUBSTANCES
Extracted: chlortetracycline, oxytetracycline, tetracycline

Also analyzed: demeclocycline

KEY WORDS
cow; liver; pig; kidney; muscle; chicken; metal chelate affinity chromatography; MCAC; SPE

REFERENCE
Croubels,S.M.; Vanoosthuyze,K.E.I.; Van Peteghem,C.H. Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg, *J.Chromatogr.B*, **1997**, *690*, 173–179.

SAMPLE
Matrix: milk
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM
Retention time: 13.9

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, minocycline, oxytetracycline, tetracycline

KEY WORDS
metal chelate affinity chromatography; cow; SPE

REFERENCE
Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with saturated aqueous disodium EDTA. Blend 5 g tissue with two 20 mL portions and one 10 mL portion of 100 mM pH 4.0 disodium EDTA-McIlvaine buffer at high speed, centrifuge at 850 g for 5 min each time. Combine the supernatants, centrifuge at 850 g for 15 min, filter. Add the filtrate to the SPE cartridge, wash with 20 mL water, air-dry by aspiration

for 5 min, elute with 10 mL ethyl acetate followed by 20 mL MeOH:ethyl acetate 5:95, evaporate the eluate to dryness under reduced pressure at 30°, dissolve the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK Gel Super Octyl (Tosoh)
Mobile phase: MeCN:0.05% aqueous trifluoroacetic acid 20:80
Flow rate: 0.5
Injection volume: 50
Detector: MS, Finnigan MAT TSQ 7000 Triple-Stage Quadrupole, electrospray voltage 4.5 kV, gas sheath flow 483 kPa nitrogen, collision gas argon, collision offset -25 V, m/z 445

CHROMATOGRAM

Retention time: 10.8

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline, tetracycline

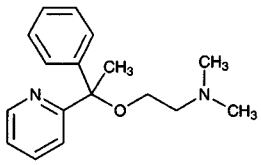
KEY WORDS

cow; SPE; kidney; liver; muscle

REFERENCE

Oka,H.; Ikai,Y.; Ito,Y.; Hayakawa,J.; Harada,K.-.; Suzuki,M.; Odani,H.; Maeda,K. Improvement of chemical analysis of antibiotics. XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *693*, 337–344.

Doxylamine



Molecular formula: $C_{17}H_{22}N_2O$
Molecular weight: 270.37
CAS Registry No.: 469-21-6, 562-10-7 (succinate)
Merck Index: 3497
Lednicer No.: 1 44

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 10 mL 300 mM NaOH + 5 mL dichloromethane, agitate in a rocking shaker at a slow speed for 15 min, centrifuge, remove the organic layer, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 µL IS solution, vortex, centrifuge, inject an 80 µL aliquot. (Prepare the IS solution as follows. 20 mg Dextroamphetamine sulfate + 10 mL 300 mM NaOH + 20 mL dichloromethane, shake for 5 min, repeat the extraction. Combine the organic layers and make up to 50 mL with dichloromethane, dilute a 5 mL aliquot to 50 mL with dichloromethane, use this solution.)

HPLC VARIABLES

Column: 300 × 3.9 10 µm µPorasil
Mobile phase: Chloroform:MeCN:buffer 80:10:10 (Buffer was MeOH:ammonium hydroxide: ammonium chloride 57:2:1.)
Flow rate: 1.5
Injection volume: 80
Detector: UV 254

CHROMATOGRAM

Retention time: 14.6
Internal standard: dextroamphetamine (12.1)
Limit of detection: 5 ng/mL

KEY WORDS

plasma; pharmacokinetics; normal phase

REFERENCE

Kohlhof,K.J.; Stump,D.; Zizzamia,J.A. Analysis of doxylamine in plasma by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 961–962.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8

Injection volume: 50
Detector: UV 261

CHROMATOGRAM

Retention time: 4.57
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; ácebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wave-

length for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.147

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.9

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, cyclizine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS

tablets; syrups; elixirs; injections

REFERENCE

Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 35:65 containing 300 mM KCl and 300 mM ammonium chloride, adjusted to pH 3.0 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 261

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 5.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, peri-

cyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.03

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 98:2
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: k' 1.80 (of first (+) enantiomer)

KEY WORDS

chiral; α 1.27

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 2.215, 2.376

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993,** *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 209, 269

CHROMATOGRAM
Retention time: 1.8
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 60:35:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1

Injection volume: 20
Detector: UV 262

KEY WORDS
chiral; α = 1.07 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 2 mL urine to 6.0, extract three times with 10 mL aliquots of dichloromethane, evaporate to dryness under reduced pressure, reconstitute in MeOH, inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 50 µm Supelco LC-CN
Column: 250 × 4.6 5 µm Supelco LC-CN
Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 5:95 containing 20 mM triethylamine, pH 7.3. B was MeOH:10 mM KH_2PO_4 95:5 containing 20 mM triethylamine, pH 7.3. A:B 100:0 for 10 min, to 0:100 over 2 min, maintain at 0:100 for 18 min, return to initial conditions over 2 min, re-equilibrate for 8 min.
Flow rate: 1
Injection volume: 100
Detector: UV 254 or radioactivity

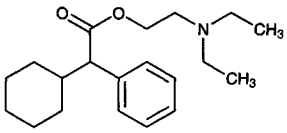
CHROMATOGRAM
Retention time: 21.5

OTHER SUBSTANCES
Extracted: metabolites, degradation products

KEY WORDS
monkey

REFERENCE
Holder,C.L.; Korfmacher,W.A.; Rushing,L.G.; Thompson,H.C.,Jr.; Slikker,W.,Jr.; Gosnell,A.B. Formation of artifactual metabolites of doxylamine following acid hydrolysis, *J.Chromatogr.*, **1987**, *419*, 113–122.

Drofenine



Molecular formula: $C_{20}H_{31}NO_2$
Molecular weight: 317.47
CAS Registry No.: 1679-76-1, 548-66-3 (HCl)
Merck Index: 3501

SAMPLE
Matrix: solutions

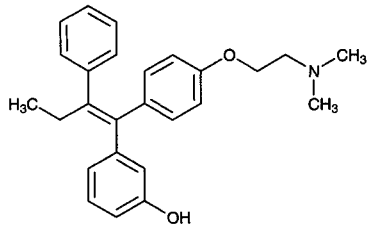
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 250 × 4 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 65:35 (Buffer was 20 mM sodium acetate containing 0.59% di-n-butylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 260

CHROMATOGRAM
Retention time: k' 3.72

OTHER SUBSTANCES
Simultaneous: cyclohexanephenylacetic acid

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1347–1357.

Droloxifene



Molecular formula: $C_{26}H_{29}NO_2$
Molecular weight: 387.52
CAS Registry No.: 82413-20-5
Merck Index: 3502

SAMPLE

Matrix: blood
Sample preparation: Mix serum with an equal volume of MeCN, mix, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 3 μm ODS Hypersil
Mobile phase: MeCN:water 66:34 containing 3 mM acetic acid and 2 mM diethylamine
Flow rate: 1
Injection volume: 50
Detector: F ex 260 em 360 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID knitted PTFE coil irradiated with UV light at 254 nm to the detector.

CHROMATOGRAM

Retention time: 18.8
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, atenolol, captopril, clodronate, dexamethasone, dextropropoxyphene, diazepam, doxycycline, econazole, enoxaparin, felodipine, flunitrazepam, furosemide, glibenclamide, indomethacin, insulin, isosorbide mononitrate, megestrol acetate, metoclopramide, mianserin, morphine, nitroglycerin, oxazepam, perphenazine, phenytoin, pivmecillinam, prochlorperazine, promethazine, ranitidine, tamoxifen

KEY WORDS

post-column reaction; post-column photochemical derivatization; serum; pharmacokinetics

REFERENCE

Lien,E.A.; Anker,G.; Lonning,P.E.; Ueland,P.M. Determination of droloxifene and two metabolites in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 259–265.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut benzenesulfonic acid (SCX) SPE cartridge with 1 mL MeOH:30% ammonium hydroxide 96.5:3.5, 1 mL MeOH, and 1 mL 1% acetic acid at 1 mL/min. Add 500 μL 1% acetic acid, 200 μL 2 ng/mL IS in mobile phase, and 200 μL plasma or serum sequentially to the SPE cartridge, wash with 1 mL water, wash with 1 mL MeOH, elute with 1 mL MeOH:30% ammonium hydroxide 96.5:3.5. Evaporate the eluate to dryness under a stream of nitrogen at 50° or reduced pressure at 75°, reconstitute with 100 μL mobile phase, vortex, centrifuge at 1000 g for 30 s, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm C18 (Rainin)
Mobile phase: MeCN:50 mM sodium phosphate buffer 45:55, adjusted to pH 3.5 with phosphoric acid

Column temperature: 40
Flow rate: 2
Injection volume: 80
Detector: F ex 260 em 375 following post-column photolysis. The column effluent passed through a 3.1 m × 0.25 mm i.d. PTFE coil, where it was irradiated by a Beam Boost 254 nm photochemical reaction lamp, to the detector.

CHROMATOGRAM

Retention time: 3.1
Internal standard: (E)-α-[p-[2-(diethylamino)ethoxy]phenyl]-α'-ethyl-3-stilbenol (K 21.089 E, Klinge Pharma, Munich) (4.8)
Limit of detection: 10 pg/mL
Limit of quantitation: 25 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

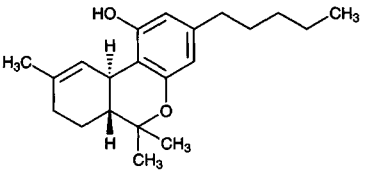
KEY WORDS

rat; monkey; human; plasma; serum; post-column reaction; pharmacokinetics; SPE; post-column photochemical derivatization

REFERENCE

Tess,D.A.; Cole,R.O.; Toler,S.M. Sensitive method for the quantitation of droloxifene in plasma and serum by high-performance liquid chromatography employing fluorimetric detection, *J.Chromatogr.B*, **1995**, *674*, 253–260.

Dronabinol



Molecular formula: $C_{21}H_{30}O_2$
Molecular weight: 314.47
CAS Registry No.: 1972-08-3
Merck Index: 9349
Lednicer No.: 1 394; 4 209

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 200 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 2 mL 8 M urea, vortex for 5 s, add 2 mL MeOH, vortex for 5 s, add to SPE cartridge, pass through within 2-3 min, rinse sample tube with 2 mL water:MeOH:8 M urea 1:2:2 and add rinse to SPE cartridge, wash with 2 mL MeOH: water 1:1, wash with 1 mL 200 mM HCl, wash with 1 mL MeOH:water 1:1, wash with 1 mL 10 mM NaOH, wash with 3 mL MeOH:water 1:1, centrifuge for 10 min to remove remaining fluid, elute with 0.5 mL diethyl ether. Evaporate eluate to dryness at 40°, dissolve residue in 100 μL MeOH, vortex for 5 s, inject a 10 μL aliquot. (Purify 8 M urea by passing 50 mL through an activated 3 mL 500 mg Bond-Elut C18 SPE cartridge.)

HPLC VARIABLES

Guard column: 10 × 2.1 40 μm Chromsep pellicular C18 (Chrompack)
Column: 100 × 4.6 3 μm Chromsep C18 (Chrompack)
Mobile phase: THF:MeOH:5 mM pH 7.0 citrate buffer 7.5:68:24.5
Flow rate: 1
Injection volume: 10
Detector: E, Metrohm glassy carbon working electrode 3 mm diameter (polished daily for 1 min with 0.3 μm aluminum oxide powder), Ag/AgCl (3 M KCl) reference electrode, stainless steel auxiliary electrode, polarize working electrode for 20 min at + 960 mV then decrease to working potential of + 760 mV.

CHROMATOGRAM

Retention time: 14
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Zweipfenning,P.G.M.; Lisman,J.A.; van Haren,A.Y.N.; Dijkstra,G.R.; Holthuis,J.J.M. Determination of delta 9-tetrahydrocannabinol in plasma using solid-phase extraction and high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1988**, *456*, 83–91.

SAMPLE

Matrix: blood, saliva

Sample preparation: 1 mL Plasma, serum, or saliva + 50 μL 1.05 μg/mL 4-dodecylresorcinol in MeOH + 2 mL MeOH + 200 μL 70% perchloric acid, vortex for 30 s, centrifuge at 2000 g for 2 min. Remove the supernatant and add it to 1 mL saturated NaCl and 150 μL toluene, vortex for 30 s, centrifuge at 2000 g for 2 min, inject an aliquot of the organic phase.

HPLC VARIABLES

Column: 150 × 3.9 μBondapak C18

Mobile phase: MeOH:water 22.5:77.5 containing 100 mM sodium monochloroacetate and 25 mM monochloroacetic acid (After each 12 analyses wash the column with MeOH at 3 mL/min for 15 min then re-equilibrate with mobile phase at 3 mL/min for 15 min.)
Flow rate: 3
Detector: E, BAS Model LC-4B, glassy carbon working electrode at + 0.90 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 5.67
Internal standard: 4-dodecylresorcinol (7.05)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, impurities

KEY WORDS

plasma; serum

REFERENCE

Thompson,L.K.; Cone,E.J. Determination of delta 9-tetrahydrocannabinol in human blood and saliva by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.*, **1987**, *421*, 91–97.

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 1-2 mL Plasma + 10-20 μL (?) 55 μg/mL cannabinol + 5 mL MeOH, mix vigorously at 0°, centrifuge at 0° at 1000 g for 5 min, suspend the white gel-like precipitate in 3 mL ice-cold MeOH, centrifuge. Combine the supernatants and evaporate them to 2 mL under a stream of air at 65°, cool in ice-water for 10-20 min, centrifuge at 0° at 1000 g for 5 min. Combine the supernatants and add 2 mL dichloromethane, vortex, add 5 mL hexanes, vortex, centrifuge at 500 g for 5 min, remove the upper organic layer, extract the lower MeOH/water layer with 2 mL dichloromethane and 5 mL hexanes. Combine the upper organic layers and evaporate them to dryness under a stream of air at 65°, reconstitute the residue in 1 mL hexanes, evaporate to dryness under reduced pressure, reconstitute the residue in 200 μL hexanes, evaporate to dryness under reduced pressure, reconstitute with 20 μL MeOH (warm slightly if necessary), add 100 μL acetone, mix vigorously, cool in ice-water for 10 min, centrifuge at 0° at 1000 g for 5 min. Remove the supernatant and add it to 75 μL EtOH, dry under reduced pressure, reconstitute with 100 μL MeOH, inject a 75 μL aliquot. Tissue. Homogenize (tissue grinder mortar) 500 mg mouse brain with 3 mL ice-cold MeOH and 10-20 μL 55 μg/mL cannabinol at 0°, centrifuge at 0° at 1000 g for 5 min, suspend the pellet in 3 mL ice cold MeOH, centrifuge at 0° at 1000 g for 5 min. Combine the supernatants and evaporate them to 1.5 mL under a stream of air at 65°, cool in ice-water for 10-20 min, centrifuge at 0° at 1000 g for 5 min, suspend the pellet in 500 μL ice-cold MeOH:water 80:20, centrifuge. Combine the supernatants and add 2 mL dichloromethane, vortex, add 1 mL water, vortex, add 5 mL hexanes, vortex, centrifuge at 500 g for 5 min, remove the upper organic layer, extract the lower MeOH/water layer with 2 mL dichloromethane and 5 mL hexanes. Combine the upper organic layers and evaporate them to dryness under a stream of air at 65°, reconstitute the residue in 1 mL hexanes, evaporate to dryness under reduced pressure, reconstitute the residue in 200 μL hexanes, evaporate to dryness under reduced pressure, reconstitute with 20 μL MeOH (warm slightly if necessary), add 100 μL acetone, mix vigorously, cool in ice-water for 10 min, centrifuge at 0° at 1000 g for 5 min. Remove the supernatant and add it to 75 μL EtOH, dry under reduced pressure, reconstitute with 100 μL MeOH, inject a 75 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 3.9 37-50 μm μBondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:water 85:15
Flow rate: 1.5
Injection volume: 75
Detector: UV 490 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 4 m × 0.8 mm PTFE coil to the detector. (Prepare the reagent by dissolving 1 g Fast Blue Salt B in 160 mL water, adding 200 mL MeOH, and adding 40 mL 10% sodium nitrite in water. Stir for 30 min, filter (Whatman GF/C), filter (0.45 μm Gelman Metricel GA-6), discard after 5 h. Protect from light. At the end of each day flush the post-column reaction system with meOH: water 50:50, acetone, and MeOH:water 50:50.)

CHROMATOGRAM
Retention time: 7
Internal standard: cannabinol (6)
Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: 11-hydroxytetrahydrocannabinol

KEY WORDS
post-column reaction; treat glassware with Dri-Film SC-87 (Pierce); mouse; plasma; brain

REFERENCE
Borys,H.K.; Karler,R. Post-column derivatization procedure for the colorimetric analysis of tissue cannabinoids separated by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *205*, 303–323.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1 mmole compound, 4 mmole imidazole, and 2 mmole t-butyldimethylsilyl chloride in 500 μL DMF, heat on a steam bath for 1 h, add 2 mL 10% NaOH, extract 3 times with 2 mL portions of petroleum ether bp (30-60). Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness.

HPLC VARIABLES
Column: 305 mm × 6.4 mm (o.d.) 10 μm μBondapak C18
Mobile phase: MeOH:water 90:10
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 16.2

OTHER SUBSTANCES
Simultaneous: cannabidiol, cannabinol
Interfering: delta^8-tetrahydrocannabinol

KEY WORDS
derivatization

REFERENCE
Knaus,E.E.; Coutts,R.T.; Kazakoff,C.W. The separation, identification, and quantitation of cannabinoids and their t-butyldimethylsilyl, trimethylsilylacetate, and diethylphosphate derivatives using high-pressure liquid chromatography, gas-liquid chromatography, and mass spectrometry, *J.Chromatogr.Sci.*, **1976**, *14*, 525–530.

SAMPLE
Matrix: formulations

Sample preparation: Dilute with EtOH to a dronabinol concentration of 5-10 mg/mL, add 100 μL of this solution to 600 μL 150 μg/mL tetraphenylethylene in EtOH, inject a 10 μL aliquot of this solution. (Dilute samples in sesame oil with n-butanol.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeOH:water 78:22
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 20
Internal standard: tetraphenylethylene (24)

OTHER SUBSTANCES

Simultaneous: impurities, degradation products

REFERENCE

Flora,K.P.; Cradock,J.C.; Davignon,J.P. Determination of delta 9-tetrahydrocannabinol in pharmaceutical vehicles by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *206*, 117–123.

SAMPLE

Matrix: formulations
Sample preparation: Cut capsules with a scalpel, dissolve contents in absolute EtOH so that the concentration of dronabinol is about 150 μg/mL, add dodecanophenone, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS + 150 × 4.6 3 μm Spherisorb ODS II
Mobile phase: MeCN:1% acetic acid 85:15
Flow rate: 0.5
Injection volume: 30
Detector: UV 220 and UV 280

CHROMATOGRAM

Retention time: 35
Internal standard: dodecanophenone

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

capsules

REFERENCE

Ray,G.; Crook,M.; West,N.; Kwoka,M.; Rehagen,G.; Cox,J.; Murrill,E.; Flora,K. Comparison of the analysis of delta 9-tetrahydrocannabinol capsules by high-performance liquid chromatography and capillary gas chromatography, *J.Chromatogr.*, **1984**, *317*, 455–462.

SAMPLE

Matrix: plants
Sample preparation: 100 mg Leaves or 50 mg resin or oil + 1 mL MeOH:chloroform 90:10, sonicate for 15 min, , filter. Dilute 100 μL filtrate with 300 μL MeOH, inject a 1 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 3 μm Spherisorb ODS-1
Column: 200 × 2 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was 8.64 g/L 85% orthophosphoric acid in water. B was MeCN. A:B from 53:47 to 40:60 over 38 min, to 30:70 over 10 min, to 53:47 over 2 min, re-equilibrate for 10 min. (Wash column with MeCN after use.)
Column temperature: 40
Flow rate: 0.2
Injection volume: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 40
Limit of quantitation: 25 ng

OTHER SUBSTANCES
Simultaneous: related compounds

KEY WORDS
leaves; resin; oil

REFERENCE
Lehmann,T.; Brenneisen,R. High performance liquid chromatographic profiling of cannabis products, *J.Liq.Chromatogr.*, **1995**, *18*, 689–700.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 305 mm × 6.4 mm (o.d.) 10 μm μBondapak C18
Mobile phase: MeOH:water 75:25
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 18.4

OTHER SUBSTANCES
Simultaneous: cannabidiol, cannabinol, delta^{8}-tetrahydrocannabinol

REFERENCE
Knaus,E.E.; Coutts,R.T.; Kazakoff,C.W. The separation, identification, and quantitation of cannabinoids and their t-butyldimethylsilyl, trimethylsilylacetate, and diethylphosphate derivatives using high-pressure liquid chromatography, gas-liquid chromatography, and mass spectrometry, *J.Chromatogr.Sci.*, **1976**, *14*, 525–530.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Mix 50 mg tissue with 2.5 mL 280 ng/mL 4-dodecylresorcinol in MeOH using a Teflon pestle, sonicate the homogenates using a Soniprep 150 ultrasonic

disintegrator (MSE Scientific Instruments, Sussex), fitted with a probe assembly with a 3.0 mm diameter tip operated at a 23 μm amplitude. Maintain the homogenates in a ice-bath and subject to three 2 min sonication intervals. Centrifuge the homogenate at 15000 g at 4° for 30 min, evaporate the supernatant to dryness and reconstitute the residue in 5 mL hexane:ethyl acetate 70:30, wash with three 2.5 mL aliquots of 50 mM sulfuric acid, centrifuge at 3000 g at 4°. Evaporate a 4.0 mL aliquot of the organic phase to dryness using a centrifugal evaporator. Reconstitute the residue in 350 μL mobile phase:MeOH 25:10, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Newguard RP-18 (Brownlee, Santa Clara, CA)
Column: 300 × 4 10μm μBondapak C18
Mobile phase: MeOH:MeCN:10 mM sulfuric acid 21:24:55
Column temperature: 22
Flow rate: 3
Injection volume: 50
Detector: E, Bioanalytical Systems LC-4B dual-electrode, TL-5A glassy carbon working electrode (1.2 V), Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 5.5
Internal standard: 4-dodecylresorcinol (8.5)
Limit of detection: 1.5 ng

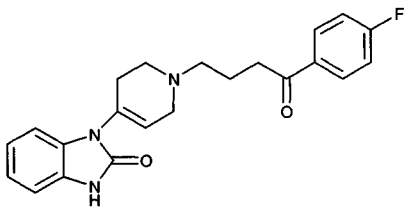
KEY WORDS

brain; rat; pharmacokinetics

REFERENCE

Nyoni,E.C.; Sitaram,B.R.; Taylor,D.A. Determination of delta9-tetrahydrocannabinol levels in brain tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1996**, *679*, 79–84.

Droperidol



Molecular formula: $C_{22}H_{22}FN_3O_2$
Molecular weight: 379.43
CAS Registry No.: 548-73-2
Merck Index: 3505
Lednicer No.: 1 308

SAMPLE

Matrix: blood
Sample preparation: Basify plasma containing flurazepam with 500 mM KOH, extract with diethyl ether:heptane 90:10. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 mm long 5 μm Spherisorb CN
Mobile phase: MeCN:EtOH:50 mM pH 2.5 phosphate buffer 20:15:65
Detector: UV 200

CHROMATOGRAM

Retention time: 3.90
Internal standard: flurazepam (4.86)
Limit of quantitation: 1 ng/mL

KEY WORDS

plasma

REFERENCE

James,J.; Lowe,D.; Karnes,H.T. Determination of histamine from plasma using derivatization with naphthalene-2,3-dicarboxaldehyde and HPLC with fluorescence detection, *Pharm.Res.*, **1992**, *9*, S21.

SAMPLE

Matrix: blood
Sample preparation: Condition a Supelclean LC-18 SPE cartridge (Supelco) with two 2 mL aliquots of MeOH, 2 mL water, and 2 mL 50 mM pH 6.9 ammonium acetate. add 1 mL plasma, wash with three 1 mL portions of 150 mM NaCl, wash with 50 μL MeOH, elute with 2 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN, filter (0.45 μm), inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 150 × 3.9 5 μm Novapak C18
Mobile phase: MeCN:10 mM pH 6.7 ammonium acetate 45:55
Flow rate: 1
Injection volume: 10
Detector: UV 250

CHROMATOGRAM

Retention time: 4.5
Internal standard: nitrazepam (2.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: flunitrazepam

Noninterfering: atropine, phenoperidine, pancuronium

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Guichard,J.; Panteix,G.; Dubost,J.; Baltassat,P.; Roche,C. Simultaneous high-performance liquid chromatographic assay of droperidol and flunitrazepam in human plasma. Application to haemodilution blood samples collected during clinical anaesthesia, *J.Chromatogr.*, **1993**, *612*, 269–275.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 µL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 µm Spherisorb cyano
Column: 250 × 4.6 5 µm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 10.9
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES
Extracted: chlorpromazine, clomipramine, cyamemazine, desipramine, flunitrazepam, haloperidol, imipramine, pipamperone, risperidone, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 246

CHROMATOGRAM

Retention time: 4.65
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone:chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pack C18

Mobile phase: MeOH:50 mM ammonium acetate 75:25 (Mix column effluent with 50 mM ammonium acetate pumped at 0.5 mL/min.)

Flow rate: 0.6

Injection volume: 10

Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, MAT TSP-2 interface, thermospray, selective reaction monitoring m/z 380-165, collision offset -25 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 μA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM

Retention time: 4.10

Limit of detection: 10 pg

OTHER SUBSTANCES

Extracted: benperidol, dextromoramide, haloperidol, methadone, penfluridol, pimozide, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS

SPE; LC/MS

REFERENCE

Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 10.88

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 202.8

CHROMATOGRAM

Retention time: 21.18

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Dilute 1:1, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS

Mobile phase: MeOH:20 mM pH 6.8 phosphate buffer 65:35

Flow rate: 1.5

Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: degradation products, methylparaben, propylparaben

KEY WORDS
stability-indicating; injections

REFERENCE
Dolezalová,M. Separation and determination of droperidol, methyl- and propylparaben and their degradation products by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *286*, 323–330.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetra-

zine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 8.63 (A), 4.71 (B)

OTHER SUBSTANCES

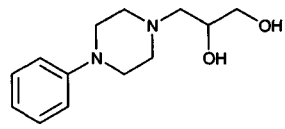
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, pro-

methazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Dropropizine



Molecular formula: $C_{13}H_{20}N_2O_2$
Molecular weight: 236.31
CAS Registry No.: 17692-31-8, 99291-24-4 (S-form)
Merck Index: 3507

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 7.243

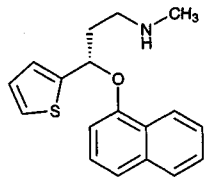
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Duloxetine



Molecular formula: $C_{18}H_{19}NOS$
Molecular weight: 297.42
CAS Registry No.: 116539-59-4, 136434-34-9 (HCl)
Merck Index: 3518

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 10 sodium carbonate buffer + 200 µL 100 ng/mL IS + 6 mL hexane:isopropanol 98:2, shake mechanically for 45 min, centrifuge at 825 g for 10 min, freeze in MeOH/dry ice. Remove the organic layer and evaporate it to dryness at 42°, reconstitute the residue in 2 mL acetone, add 150 µL 100 mM pH 10 sodium carbonate buffer, add 75 µL 500 µg/mL dansyl chloride, heat at 55° for 30 min. Evaporate to dryness under a stream of nitrogen at 42°, reconstitute the residue in 200 µL mobile phase, filter, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 12.5 × 4 5 µm Zorbax ODS
Column: 250 × 4.6 5 µm Primesphere 5 C18 (Phenomenex)
Mobile phase: MeCN:50 mM ammonium acetate 79:21
Flow rate: 2
Injection volume: 100
Detector: F ex 285 em 525

CHROMATOGRAM

Retention time: 9.3
Internal standard: N-methyl-3-(1-naphthalenyloxy)-3-phenylpropanamine (Eli Lilly 113821) (10.9)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, desmethyl duloxetine

KEY WORDS

derivatization; plasma; silanize glassware; pharmacokinetics

REFERENCE

Johnson,J.T.; Oldham,S.W.; Lantz,R.J.; Delong,A.F. High performance liquid chromatographic method for the determination of duloxetine and desmethyl duloxetine in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1631–1641.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 250 × 4.6 5 µm Zorbax SB-CN
Mobile phase: Gradient. A was MeCN:25 mM pH 3.0 potassium dihydrogen phosphate buffer 20:80. B was MeCN:25 mM pH 3.0 potassium dihydrogen phosphate buffer 75:25. A:B from 100:0 to 70:30 over 15 min, to 0:100 over 12 min
Flow rate: 1
Detector: UV 215

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES
Extracted: impurities

KEY WORDS
pellets; stability-indicating

REFERENCE
Jansen,P.J.; Oren,P.L.; Kemp,C.A.; Maple,S.R.; Baertschi,S.W. Characterization of impurities formed by interaction of duloxetine HCl with enteric polymers hydroxypropyl methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate, *J.Pharm.Sci.*, **1998**, *87*, 81–85.

SAMPLE
Matrix: formulations

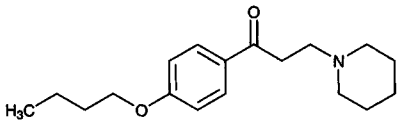
HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax SB-CN
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 20:80:0.05. B was MeCN:water:trifluoroacetic acid 75:25:0.05. A:B from 100:0 to 70:30 over 15 min, to 0:100 over 12 min
Flow rate: 1
Detector: MS, Fisons Instruments VG Quattro triple quadrupole, electrospray, positive ion mode, m/z 297

OTHER SUBSTANCES
Extracted: impurities

KEY WORDS
pellets; stability-indicating

REFERENCE
Jansen,P.J.; Oren,P.L.; Kemp,C.A.; Maple,S.R.; Baertschi,S.W. Characterization of impurities formed by interaction of duloxetine HCl with enteric polymers hydroxypropyl methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate, *J.Pharm.Sci.*, **1998**, *87*, 81–85.

Dyclonine



Molecular formula: $C_{18}H_{27}NO_2$
Molecular weight: 289.42
CAS Registry No.: 586-60-7, 536-43-6 (HCl)
Merck Index: 3523

SAMPLE

Matrix: formulations
Sample preparation: 1 mL Sample + 18 mL THF:MeCN 30:70, adjust the pH to 6.0 with 1 M ammonium hydroxide, make up to 25 mL with 100 mM pH 6.0 ammonium acetate buffer, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:THF:pH 6.0 ammonium acetate buffer 46:9:45
Flow rate: 2
Injection volume: 20
Detector: UV 282

CHROMATOGRAM

Retention time: 5.8

OTHER SUBSTANCES

Simultaneous: degradation products

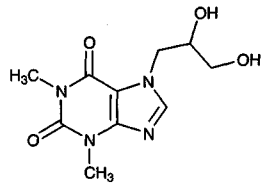
KEY WORDS

stability-indicating; oral spray; gel

REFERENCE

Bhagat,H.R.; Bhargava,H.N.; Williams,D.A. High-performance liquid chromatographic determination of dyclonine hydrochloride in the presence of its degradation products, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 441–446.

Dyphylline



Molecular formula: $C_{10}H_{14}N_4O_4$
Molecular weight: 254.25
CAS Registry No.: 479-18-5
Merck Index: 3529

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 0.2 mg/mL β-hydroxyethyltheophylline in buffer + 100 µL 40% aqueous trichloroacetic acid, vortex for 30 s, let stand for 5 min, centrifuge at 2000 g for 15 min, inject a 25 µL aliquot of the supernatant. (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)

HPLC VARIABLES

Column: 10 µm µBondapak C18
Mobile phase: MeCN:buffer 6:94 (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)
Column temperature: 40
Flow rate: 2
Injection volume: 25
Detector: UV 274

CHROMATOGRAM

Retention time: 7.5
Internal standard: β-hydroxyethyltheophylline (9)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, theobromine

KEY WORDS

plasma

REFERENCE

Valia,K.H.; Hartman,C.A.; Kucharczyk,N.; Sofia,R.D. Simultaneous determination of dyphylline and theophylline in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *221*, 170–175.

SAMPLE

Matrix: blood
Sample preparation: 50 µL Serum + 50 µL 15 µg/mL β-hydroxyethyltheophylline in MeCN + 2 mL chloroform:isopropanol 95:5, mix for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.8
Internal standard: β-hydroxyethyltheophylline (5.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: caffeine
Simultaneous: acetaminophen, aspirin, salicylic acid, procainamide, N-acetylprocainamide
Noninterfering: benzoic acid
Interfering: ampicillin, theophylline (separated with MeCN:buffer 8:92)

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 50 μL 30 μg/mL theophylline in water, vortex, add 1.5 g anhydrous sodium sulfite, add 2.5 mL chloroform:MeOH 90:10, shake vigorously for 30 s (work quickly to avoid forming a cake of sodium sulfite), centrifuge at 1000 g for 5 min. Remove the organic layer, filter (Whatman No. 1 paper pre-wetted with chloroform), rinse filter with 500 μL chloroform, evaporate the filtrate under a stream of nitrogen at 40°. Take up the residue in 100 μL dichloroethane and add it to 100 μL 100 mM ammonium carbonate, vortex for 10 s, centrifuge at 1000 g for 5 min, inject a 10 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-8
Mobile phase: MeOH:buffer 25:75 (Buffer was 1.5 mL 1 M KH_2PO_4 in 750 mL water, adjust to pH 3.0 with 900 mM perchloric acid.)
Flow rate: 2
Injection volume: 10
Detector: UV 275

CHROMATOGRAM
Retention time: 3
Internal standard: theophylline (4.5) (alternatively, β-hydroxypropyltheophylline (3.7))
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: theobromine, caffeine
Noninterfering: acetaminophen, acetazolamide, albuterol, amitriptyline, amobarbital, carbamazepine, diazoxide, disopyramide, ethosuximide, isoprenaline, nitrazepam, nortriptyline, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, salicylic acid, sulthiame

KEY WORDS
serum; pharmacokinetics

REFERENCE
Paterson,N. High-performance liquid chromatographic method for the determination of diprophylline in human serum, *J.Chromatogr.*, **1982**, *232*, 450–455.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Serum or plasma + 200 μL 100 mM pH 7.0 phosphate buffer + 3 mL 0.5 μg/mL 8-chlorotheophylline in isopropanol, stir at 40000 rpm for 5 s using dental micromotor with a PTFE mixing head, centrifuge at 3500 g for 2 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 3.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:10 mM pH 5.2 sodium acetate buffer 6:3:91
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 274

CHROMATOGRAM

Retention time: 6.7
Internal standard: 8-chlorotheophylline (8.85)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, proxyphylline, paraxanthine
Simultaneous: cefoxitin
Noninterfering: carbenicillin, cefoperazone, cephacetril, heparin, penicillin G, phenytoin, phenobarbital

KEY WORDS

plasma; serum; pharmacokinetics

REFERENCE

Wenk,M.; Eggs,B.; Follath,F. Simultaneous determination of diprophylline, proxyphylline and theophylline in serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *276*, 341–348.

SAMPLE

Matrix: blood

Sample preparation: Centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm internal surface reversed phase Pinkerton, silica derivatized with glycine-phenylalanine-phenylalanine (Regis) (periodically reverse the column)
Mobile phase: 100 mM pH 6.8 phosphate buffer
Flow rate: 0.3
Injection volume: 10
Detector: UV 275

CHROMATOGRAM

Retention time: 8.43
Limit of detection: <1000 ng/mL

OTHER SUBSTANCES

Extracted: doxofylline, theophylline, caffeine
Noninterfering: acetaminophen, amitriptyline, amphetamine, atropine, benzoylecgonine, benztropine, caffeine, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, chlorprothixene, cimetidine, cocaine, codeine, dextromethorphan, diazepam, diphenhy-

dramine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, flurazepam, glutethimide, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, nicotine, nordiazepam, nortriptyline, orphenadrine, papaverine, pentazocine, phenacetin, phencyclidine, phenmetrazine, phenolphthalein, phentermine, phenylpropanolamine, phenytoin, prazepam, procainamide, procaine, propoxyphene, propranolol, protriptyline, pseudoephedrine, pyrilamine, quinine, salicylamide, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, trimethobenzamide, trimethoprim, tripelennamine

KEY WORDS

plasma; serum; direct injection

REFERENCE

Tagliaro,F.; Dorizzi,R.; Frigerio,A.; Marigo,M. Non-extraction HPLC method for simultaneous measurement of dyphylline and doxofylline in serum, *Clin.Chem.*, **1990**, *36*, 113–115.