
HPLC METHODS FOR PHARMACEUTICAL ANALYSIS

George Lunn and Norman R. Schmuft



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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). For each compound various techniques are described in sufficient detail that the analyst can replicate the procedure without reference to the original publication. Since detailed procedures for the same drug are listed together, it is very easy for the researcher to combine features of different methods, e.g., the extraction procedure from one paper and the chromatographic procedure from another paper, to provide methods tailored to the researcher's requirements. In addition to the detailed procedures, bibliographies are provided listing other references. These references are annotated so that the reader can rapidly determine those procedures likely to be of the most utility. In the current volume, we have listed procedures for the analysis of the most commonly used drugs in the United States.^{1,2} In future volumes we hope to cover the remaining drugs used for medical and veterinary purposes.

The impetus for writing this book was the realization that there was no single volume listing analytical procedures and that there was, in particular, no ready source of information on the analysis of drugs in biological fluids other than the original literature. Although a number of methods are in common use for the analysis of pharmaceuticals, HPLC may be regarded as the "gold standard"³ and so we have decided to concentrate on this procedure. For example, HPLC assays of antibiotics have advantages such as specificity, better accuracy and precision, and wider availability of equipment, over other methods. Thus, HPLC procedures are gradually coming to replace other techniques.⁴

Although the universal penetration of computers has led to readily available laboratory-based searches of the literature, this resource is not exploited as much as it might be. An FDA inspector has stated⁵ that many pharmaceutical firms, when questioned about deficiencies in this area, admit to never having performed a literature search for HPLC methods. 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 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 without having to

consult the original literature. For many compounds the number of analytical procedures is so large that it is not possible to fully abstract all of them. For this reason we have added annotated bibliographies so that the researcher can rapidly identify a relevant paper without having to locate and evaluate a large number of irrelevant procedures.

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

We would like to thank the staff of the NCI-Frederick Cancer Research and Development Center Scientific Library for their help with this project. In particular, we would like to thank Pat Kuhns-Kelly for her diligent assistance with the computer searches and Ethel Armstrong for her indefatigable efforts in obtaining many of the more obscure references via Interlibrary Loan. The use of the NIH Library, National Institutes of Health, Bethesda, MD, is also greatly appreciated. We would also like to thank Perry King of John Wiley for help with the electronic version. Special thanks are due to our editor, Betty Sun, who brought the whole project together. The initial research which later became the nucleus of this book was supported by the Division of Safety, NIH, through NCI contract NO1-CO-74102 with Program Resources, Inc. Although many people have helped with the preparation of this work, the mistakes are our own. We would appreciate hearing from anyone who has corrections, comments, or suggestions. We can be reached at 71061.2731@compuserve.com or norman.schmuff@tcs.wap.org.

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

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ABOUT THIS BOOK

SCOPE

Based on surveys of the top 200 drugs by number of prescriptions filled¹ and the top 100 drugs by dollar sales,² we selected the most commonly used drugs in the United States. Analytical procedures for these drugs are described in this book. In future volumes, we hope to cover other drugs used for medical and veterinary purposes.

After the target compounds were identified, a computer search was used to identify relevant references. In general, the computer search was conducted using Medline, 1980 to 1996, but for a few of the most common drugs the complete *Chemical Abstracts* file on Dialog was used. Tests showed that retrieval using Dialog and Medline was similar, except that references from the *Journal of Liquid Chromatography* are not included in Medline. All relevant references from the *Journal of Liquid Chromatography* (1980 to 1996) were manually added to the database.

In Medline the search strategy was:

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 (formerly *American Journal of Hospital Pharmacy*)

Analyst

Analytical Chemistry

Antimicrobial Agents and Chemotherapy

Arzneimittelforschung

Biochemical Pharmacology

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**Drug Metabolism Reviews**Farmaco**Journal of Analytical Toxicology**Journal of AOAC International* (formerly *Journal of the Association of Official Analytical Chemists*)*Journal of Chromatographic Science**Journal of Chromatography* (Part A and Part B)*Journal of Clinical Pharmacology**Journal of Forensic Sciences**Journal of Liquid Chromatography & Related Technology* (formerly *Journal of Liquid Chromatography*)*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. In general, the literature is covered from 1980 to 1996, but a few earlier references are included.

Two other books list some HPLC procedures for drug analyses. The *United States Pharmacopeia*³ lists procedures for the assay of drugs in formulations and *Official Methods of Analysis*⁴ lists some procedures for determining drug residues in food, drink, formulations, and so on. However, these books do not list procedures for determining drugs in biological fluids, raw materials, etc. Additionally, not all the procedures are HPLC procedures. Since these books are widely available, the HPLC procedures they contain are not abstracted in this book.

This book uses non-English characters that are available in extended ASCII. Other non-English characters will be represented by the closest English equivalent or will be spelled out. This is particularly important with the names of authors. For example, Bronnum, where the o has a stroke through it, will be printed as shown because an o with a stroke through it is not available in extended ASCII. On the other hand, Carratù will be printed as shown because u with an accent is available in extended ASCII (#151). A similar situation applies with Greek characters. Thus α , β , and so on, are available and will be printed as such, but a capital delta is not available and will be represented by "delta." The extended ASCII characters which are used include the following: α , β , δ , ϵ , Θ , μ , π , σ , Σ , τ , $\acute{\alpha}$, $\grave{\alpha}$, $\hat{\alpha}$, $\ddot{\alpha}$, Å , å , Ă , Ç , \acute{e} , \acute{E} , \grave{e} , \grave{e} , \ddot{e} , \acute{i} , \grave{i} , \hat{i} , \ddot{i} , \acute{n} , \acute{N} , \acute{o} , \grave{o} , \hat{o} , \ddot{o} , Ö , \acute{u} , \grave{u} , \hat{u} , \ddot{u} , Ü , \leq , \pm , \geq , and $^{\circ}$.

MONOGRAPH STRUCTURE

Each monograph is headed by the name and structure of the target compound as well as the CAS Registry Number, molecular formula, and molecular weight. At the end of the book other names, such as trade names, that are used for this compound are given with references to the relevant monograph. Note that these names may be for formulations containing salts (e.g., the hydrochloride), mixtures of the compound with other drugs, or derivatives of the compound (e.g., esters). Names of salts (which would have identical chromatographic properties) are not further identified. Names of derivatives, such as esters, which would have different chromatographic properties, are identified by placing the derivative name in parentheses. In general, the United States Adopted Name (USAN) is used for the title of each monograph, although we have sometimes used a truncated version of this name, e.g., adiphenine for adiphenine hydrochloride. Mention of other names is only for the purpose of locating the drug under its US Adopted Name. The only exceptions are isotretinoin and tretinoin, which are listed in the monograph "Retinoic Acid," some of the steroids, which are listed for convenience in the monograph "Estrogens, conjugated," hyoscyamine, which is listed in the monograph for its racemate, atropine, levonorgestrel, which is listed in the "Norgestrel" monograph, and clavulanate potassium, which is listed in the "Clavulanic Acid" monograph. Separations that include estradiol and other conjugated estrogens are dealt with in the "Estrogens, conjugated" monograph. Separations that deal only with estradiol are dealt with in the estradiol monograph. Conjugated Estrogens, USP is defined³ as "... a mixture of sodium estrone sulfate and sodium equilin sulfate, derived wholly or in part from equine urine or synthetically from estrone and equilin." This drug substance is further specified as containing a range of sodium estrone sulfate and sodium equilin sulfate as major components. Ranges of minor components, described as "... sodium sulfate conjugates ... of 17 α -dihydroequilin, ... 17 α -estradiol, ... and 17 β -dihydroequilin ...," are also specified. The current FDA Office of Generic Drugs guidance (August 21, 1991) on "Conjugated Estrogen Tablets—In Vivo Bioequivalence and In Vitro Drug release" (page 4) states that "... bioequivalence must be demonstrated with respect to the plasma concentrations of unconjugated estrone and equilin, as well as with respect to the plasma concentrations of conjugated estrone and equilin (the sulfate conjugates of total estrone and equilin)." The manufacturer states⁵ that conjugated estrogens is a mixture containing estrone, equilin, 17 α -dihydroequilin, 17 α -estradiol, equilenin, and 17 α -dihydroequilenin as salts of their sulfate esters. As the situation may change, readers are encouraged to consult the latest USP Supplement, and the latest Pharmacopeial Forum. The latest FDA guidance can be obtained from the FDA's Office of Generic Drugs, Division of Bioequivalence, HFD-650, MPN-2, 5600 Fishers Lane, Rockville, MD 20857.

In general, molecular formulae and molecular weights are given for the simplest form of the compound. Thus, although a particular drug might be used as the hydrochloride, the molecular formula and molecular weight will refer to the free base. We have listed the CAS Registry Numbers that refer to the various forms of each drug. For example, CAS Registry Numbers might be listed for free base, hydrochloride, and hydrochloride dihydrate.

At the end of the book cross references are given to the relevant abstracts in *The Merck Index*⁶ and the relevant sections in the series *Organic Chemistry of Drug*

Synthesis by Lednicer and Mitscher.⁷⁻¹¹ Much useful information, such as melting point, solubility, optical rotation, and references to reviews, can be found in *The Merck Index*. The series by Lednicer and Mitscher gives valuable information about the syntheses of various drugs, and this may be helpful in determining impurities, understanding degradation reactions, and so on.

Each monograph comprises two sections: Procedures and Annotated Bibliography. The first section presents detailed procedures which should enable anyone to reproduce the analyses. The second section lists other relevant papers, but does not give any experimental details. However, some key words are given to indicate key features of the procedure that are not referred to in the title. The intent of this section is to allow readers to determine rapidly if a paper is relevant and worth looking up in the library.

ABSTRACT STRUCTURE

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

Reference

Matrix

Sample Preparation

Guard Column

Column

Mobile Phase

Flow Rate

Injection Volume

Retention Time

Detector

Internal Standard

Limit of Detection

Limit of Quantitation

Drugs that Are Extracted under These Conditions

Drugs that Are Chromatographed Simultaneously under These Conditions

Drugs that Are Also Chromatographed under These Conditions

Drugs that Are Non-interfering

Drugs that Are Interfering

Key Words

ABSTRACT CONVENTIONS

If not otherwise indicated, the detailed procedures describe procedures carried out at ambient temperature using stainless steel columns with biological fluids from humans. In some cases, these parameters may be specified. For example, if **both**

human blood and rat blood are analyzed, **both** human and rat will be indicated in the key words section. Note that the noun is used instead of the adjective, e.g., cow **not** bovine.

Similarly, if not otherwise indicated, the procedures in the Annotated Bibliography describe conventional isocratic reverse-phase HPLC at ambient temperature using stainless steel columns with UV detection and biological fluids from humans. In some cases, these parameters may be specified. For example, if **both** normal-phase and reverse-phase techniques are used, **both** will be indicated. Notations are made only if the operating conditions were different from those specified above. For example, if a gradient or column heater was used, this fact will be noted. On the other hand, if the detector is a UV detector, no reference to the detector is made. Some annotation terms that may be used are as follows: chiral, column-switching, column temp [if other than ambient (in °C)], derivatization, gradient, matrix, metabolites (if method resolves compound and metabolites), normal phase, radiolabeled, species (if other than human), stability-indicating, and so on.

Note that the 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.

Gradients are linear and mobile phases are v/v 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)."

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.

Retention times are frequently estimated from reproduced chromatograms and so the accuracy may not be high. In particular, differences in retention times between adjacent peaks, e.g., enantiomers, may have a high margin of error.

EXTRACTION FROM BIOLOGICAL MATRICES

In this book certain terms concerning extraction from biological matrices have highly specific meanings. These terms are as follows:

Also	Compounds that can be analyzed under the same conditions as the target compound. It is not specified whether they interfere or can be extracted from the biological matrix in question.
Extracted	Compounds that can be extracted from the biological matrix in question, can be chromatographed under the same conditions, and do not interfere with the determination of the target compound.

Interfering	Compounds that interfere with the analysis of the target compound. Compounds that interfere with the chromatography of the internal standard are not listed in this category because another internal standard can always be selected or an external standard procedure can be used.
Non-interfering	Compounds that do not interfere with the analysis because no peaks appear on the chromatogram.
Simultaneous	Compounds that can be chromatographed at the same time as the target compound and do not interfere with the determination of the target compound. Note that the compound cannot necessarily be extracted from the biological matrix in question (although it may be).

It is common practice, during the validation of a method for the analysis of a drug in biological fluids, to run a variety of other drugs so as to demonstrate interference or non-interference with the extracted drug. However, these other drugs are generally analyzed as solutions in solvents rather than in biological fluids. Methods in which the drugs are analyzed as solutions in solvents are generally not listed in the monographs. However, these procedures can be found using the “simultaneous” feature of the database. In these instances, the chromatographic procedure will be valid, but the extraction procedure from the biological fluid may not necessarily be valid for this drug.

MATRICES

In an attempt to simplify searching procedures, we have made an effort to minimize the variety of terms used in the matrix heading. However, in a number of cases, the matrix is associated with various key words that can be used to narrow the search. For example, the term “formulations” has the key words tablets, creams, ointments, and injections associated with it. Thus, to find references applicable to tablets, search first for formulations under the matrix heading and then tablets under the key word heading. Note that the term “bulk” is used instead of “raw materials.” Some of the more common matrix terms and their associated key words are given below.

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

DETECTORS

The following terms are used for HPLC detectors.

Chemiluminescence

Conductivity

E electrochemical

ELSD evaporative light-scattering detector

F fluorescence

MS mass spectrometry

Radioactivity

RI refractive index

UV ultraviolet

UNITS

The units used are as follows:

column dimensions in mm (length \times internal diameter)

flow rates in mL/min

injection volume in μ L

retention time in min

temperatures in $^{\circ}$ C

wavelengths in nm

ABBREVIATIONS

α separation factor; defined by k'_2/k'_1 where k'_2 is the capacity factor of the second peak and k'_1 is the capacity factor of the first peak.

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

CE capillary electrophoresis

DMF dimethylformamide

DMSO dimethyl sulfoxide

em emission wavelength

EtOH ethanol

ex excitation wavelength

F fluorescence detection

FW formula weight

GPC gel permeation chromatography

h hour

IS internal standard

k' capacity factor; defined by $(t_R - t_0)/t_0$ where t_R is the retention time and t_0 is the column dead time

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 <i>tert</i> -butyl ether
nM	nano-molar (i.e., nano-moles/L)
RT	retention time
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

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

WORKING PRACTICES

In general, good working practice, e.g., filtering and degassing mobile phases, using in-line filters, HPLC or analytical grade materials, and high-quality water 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. A number of excellent texts¹²⁻¹⁴ discuss good working practices and procedures in HPLC and these should be consulted.

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.

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

A number of solvents used in HPLC are particularly hazardous. For example, benzene is a human carcinogen;¹⁵ chloroform,¹⁶ dichloromethane,¹⁷ dioxane,¹⁸ and carbon tetrachloride¹⁹ are carcinogenic in experimental animals; and DMF²⁰ and MTBE^{21,22} 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.²³ Disposal procedures have been described for a number of hazardous drugs and reagents²³ and recent papers describe a procedure for the hydrolysis of acetonitrile in waste solvent to the much less toxic acetic acid and ammonia.^{24,25} Recent work has shown that n-hexane is surprisingly toxic.²⁶

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

Item	Supplier
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.
μ Bondapak	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
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.

Trademark	Company
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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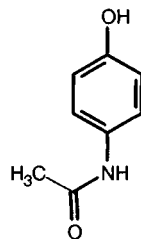
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Acetaminophen

Molecular formula: C₉H₉NO₂

Molecular weight: 151.2

CAS Registry No.: 103-90-2



SAMPLE

Matrix: bile, blood, tissue, urine

Sample preparation: Tissue. Macerate 2 g tissue with 5 mL water, add 15 mL MeCN, add 0.1 mL 1 mg/mL 2-acetamidophenol in ethanol, shake, centrifuge at 5200 g. Transfer supernatant to 50 mL tube containing 8 mL diethyl ether + 12 mL dichloromethane + 1 mL citrate buffer, vortex, proceed as in (A). Bile. 2 mL Bile + 3 mL water, add 15 mL MeCN, add 0.1 mL 1 mg/mL 2-acetamidophenol in ethanol, vortex, add 8 mL diethyl ether + 12 mL dichloromethane, vortex, centrifuge at 5200 g, proceed as in (A). Blood, urine. 5 mL Blood or urine + 15 mL acetone + 0.1 mL 1 mg/mL 2-acetamidophenol in ethanol, vortex for a few s, add 8 mL diethyl ether, vortex, centrifuge 5200 g. Transfer supernatant to 50 mL tube, add 12 mL dichloromethane, add 1 mL citrate buffer, vortex, proceed as in (A). (A). Discard lower, aqueous layer. Filter the organic layer through 3 g florisil + 8 g anhydrous sodium sulfate and wash through with 15 mL diethyl ether. Evaporate filtrate to dryness under a stream of air at 40°. Reconstitute in 5 mL MeCN:0.1 N NaH₂PO₄ 60:40 + 3 mL hexane, vortex. Remove and discard upper hexane layer, add 8 mL 20% (v/v) isopropanol in chloroform to the aqueous layer, vortex. Remove and discard the upper aqueous layer and evaporate lower layer. Reconstitute residue in MeCN:water 10:90, inject an aliquot. (Citrate buffer was saturated sodium citrate containing enough sodium tungstate (sic) to bring pH to 8. To each 1 L of diethyl ether 10 mL of water and 1 mL of citrate buffer are added.)

HPLC VARIABLES

Column: 100 × 4.6 C18 microbore

Mobile phase: MeCN:dilute phosphoric acid (1 mL 85% phosphoric acid in 140 mL water) 7:93

Column temperature: 40

Flow rate: 0.3

Detector: UV 271

CHROMATOGRAM

Retention time: 2.4

Internal standard: 2-acetamidophenol (4.9)

Limit of detection: 50 (blood, urine),200 (bile, tissue) ng/mL

OTHER SUBSTANCES

Extracted: theophylline

KEY WORDS

liver

REFERENCE

Mathis, D.F.; Budd, R.D. Extraction of acetaminophen and theophylline from post-mortem tissues and urine for high-performance liquid chromatographic analysis. *J.Chromatogr.*, **1988**, *439*, 466–469

SAMPLE

Matrix: blood

Sample preparation: 500 µL Plasma + 25 µL 40 µg/mL 3-acetamidophenol, extract with ether:dichloromethane:isopropanol 59:40:1. Remove the organic layer and evaporate it

2 Acetaminophen

to dryness under a stream of nitrogen, reconstitute the residue in 250 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 microsphere C18 (Chrompack)

Mobile phase: MeOH:50 mM pH 7.8 sodium phosphate buffer 4:96

Flow rate: 1

Detector: UV 278

CHROMATOGRAM

Internal standard: 3-acetamidophenol

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: 100 μ L Plasma + 1 μ g p-anisamide + 1 mL 500 mM pH 7.4 $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer + 1 mL ethyl acetate, shake for 10 min, centrifuge at 1300 g for 5 min. Evaporate the supernatant to dryness under reduced pressure, reconstitute the residue in 200 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4 Nucleosil 7C18

Mobile phase: MeCN:1% acetic acid 10:90

Flow rate: 1.5

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Internal standard: p-anisamide

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: sulfapyridine

KEY WORDS

plasma; dog; pharmacokinetics

REFERENCE

Sagara, K.; Mizuta, H.; Ohshiko, M.; Shibata, M.; Haga, K. Relationship between the phasic period of interdigestive migrating contraction and the systemic bioavailability of acetaminophen in dogs. *Pharm.Res.*, 1995, 12, 594-598

SAMPLE

Matrix: blood

Sample preparation: 0.5 mL Plasma + 25 μ L 40 μ g/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: metabolites, antipyrine

KEY WORDS

plasma; 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: 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 KH₂PO₄ 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 KH₂PO₄ 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: 2.09

Internal standard: 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES

Extracted: amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental

Also analyzed: acetanilide, butabarbital, butalbital, cimetidine, cyheptamide, diazoxide, diflunisal, disopyramide, ethchlorvynol, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, naproxen, nirvanol, phenacetin, phensuximide, phenylbutazone, salicylamide, sulindac, tolmetin

Noninterfering: N-acetylcysteine, N-acetylprocainamide, amikacin, ampicillin, aspirin, chlorpropamide, codeine, diphylline, gentamicin, gentisic acid, meprobamate, morphine, netilmicin, procainamide, quinidine, salicylic acid, sulfamethoxazole, tetracycline, tobramycin, trimethoprim, valproic acid, vancomycin

Interfering: oxphenylbutazone

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: Activate a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply to cartridge. Wash with 100 μ L water, elute with three 500 μ L portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 5 μ m Spherisorb ODS

Mobile phase: MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 250

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Simultaneous: ketoprofen, salicylic acid, naproxen, fenoprofen, ibuprofen, indomethacin

KEY WORDS

whole blood; SPE

REFERENCE

Moore, C.M.; Tebbett, I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis. *Forensic Sci.Int.*, **1987**, *34*, 155-158

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 75 μ L water, mix, inject a 20 μ L aliquot directly.

HPLC VARIABLES

Guard column: 23 \times 3.9 37-50 μ m Bondapak C18/Corasil

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: 50 mM pH 6.0 phosphate buffer

Flow rate: 2.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 18

Internal standard: acetaminophen

OTHER SUBSTANCES

Extracted: uric acid, oxipurinol, allopurinol

KEY WORDS

plasma; renew guard column after 50-70 injections; acetaminophen is IS

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: 50 μ L Serum + 50 μ L 100 mM HCl + 50 μ L 15 μ g/mL β -hydroxyethyltheophylline in MeCN + 3 mL ethyl acetate, 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 μ L MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: μ Bondapak 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 column with water for 20 min and MeOH for 30 min.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4.3

Internal standard: β -hydroxyethyltheophylline (5.8)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: dyphylline, theophylline, caffeine, 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: 200 μ L Serum + 200 μ L 50 μ g/mL hexobarbital in MeCN + 25 μ L glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: μ Bondapak 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: 3.3

Internal standard: hexobarbital (20.6)

Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: amobarbital, 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, propranolol, quinidine

Interfering: procainamide, phenylpropanolamine

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, CSF, gastric fluid, urine

Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m 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: 4.93

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, 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, saliva

Sample preparation: 500 μ L Saliva or plasma + 100 μ L 100 μ g/mL o-hydroxyacetanilide + 5 mL ethyl acetate, 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 50°, reconstitute the residue in 200 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4 Inertsil ODS-2

Mobile phase: MeOH:1.5% acetic acid 15:85 (plasma) or MeOH:MeCN:50 mM pH 2.5 potassium phosphate buffer 8:7:85 (saliva)

Column temperature: 50

Flow rate: 1

Injection volume: 50

Detector: UV 240

CHROMATOGRAM

Retention time: 4.7 (plasma), 4.0 (saliva)

Internal standard: o-hydroxyacetanilide (9.9 (plasma), 8.0 (saliva))

Limit of quantitation: 100 ng/mL

KEY WORDS

plasma; human; dog; pharmacokinetics

REFERENCE

Katori, N.; Aoyagi, N.; Terao, T. Estimation of agitation intensity in the GI tract in humans and dogs based on *in vitro/in vivo* correlation. *Pharm.Res.*, **1995**, *12*, 237–243

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: 900 μ L Plasma or saliva + 10 μ L 10 μ g/mL 3-acetaminophen in 30% perchloric acid, mix, centrifuge at 3000 rpm for 15 min, inject an aliquot of the supernatant

HPLC VARIABLES

Guard column: 50 \times 4.6 37-53 μ m pellicular ODS (Whatman)

Column: 250 \times 4.6 5 μ m Spherisorb ODS2

Mobile phase: MeCN:20 mM orthophosphoric acid 4:96, pH 3.5 (urine) or MeOH:MeCN:20 mM orthophosphoric acid 2:4:94, adjusted to pH 3.2 (plasma) or MeCN:20 mM orthophosphoric acid 6:94 (saliva)

Column temperature: 25

Detector: UV (wavelength not specified)

CHROMATOGRAM

Retention time: 8.3 (urine), 6.6 (plasma), 6.0 (saliva)

Internal standard: 3-acetaminophen (12.1 (urine), 9.3 (plasma), 8.3 (saliva))

Limit of detection: 200 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Al-Obaidy, S.S.; Po, A.L.W.; McKiernan, P.J.; Glasgow, J.F.T.; Millership, J. Assay of paracetamol and its metabolites in urine, plasma and saliva of children with chronic liver disease. *J.Pharm. Biomed.Anal.*, **1995**, *13*, 1033–1039

SAMPLE

Matrix: blood, urine

Sample preparation: 200 μ L Plasma or urine spiked with β -hydroxyethyltheophylline + 200 μ L 1 M tetrabutylammonium dihydrogen phosphate + 0.2 g potassium sulfate, mix 1 min, add 3 mL chloroform:isopropanol 1:1, mix 1 min, centrifuge 5 min 2500 g. Remove 2 mL of organic layer, evaporate to dryness at 35° under stream of nitrogen, take up in 200 μ L mobile phase by mixing for 1 min, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 μ m C18 (FSA Laboratory Supplies, Loughborough)

Mobile phase: MeOH:1% acetic acid containing 0.5 mM tetrabutylammonium dihydrogen phosphate and 20 mM potassium sulfate 18:82

Flow rate: 1.2

Detector: UV 254

CHROMATOGRAM

Retention time: 5

Internal standard: β -hydroxyethyltheophylline

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Kamali, F.; Herd, B. Liquid-liquid extraction and analysis of paracetamol (acetaminophen) and its major metabolites in biological fluids by reversed-phase ion-pair chromatography. *J.Chromatogr.*, **1990**, *530*, 222–225

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 750 μ g/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μ m), inject a 15 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4.5 μ m LiChrospher 100

Column: 125 \times 4.3 μ m 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: 5.7

OTHER SUBSTANCES

Simultaneous: acetylcodeine, benzocaine, 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: bulk, formulations

Sample preparation: Weigh out bulk drug, capsule contents, granules, or powders equivalent to 35 mg cephalexin, add 1 mL 2.52 mg/mL acetaminophen in MeOH:water 20:80, make up to 50 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:1.25% acetic acid 25:75

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 6

Internal standard: acetaminophen

OTHER SUBSTANCES

Simultaneous: cephalexin

KEY WORDS

capsules; granules; powder; acetaminophen is IS

REFERENCE

Hsu, M.C.; Lin, Y.-S.; Chung, H.-C. High-performance liquid chromatographic method for potency determination of cephalexin in commercial preparations and for stability studies. *J.Chromatogr.A*, **1995**, *692*, 67–72

SAMPLE

Matrix: dialysate

Sample preparation: Dialyze blood with Ringer's solution, inject a 0.5 μ L aliquot of the dialysate.

HPLC VARIABLES

Column: 14 \times 1.3 μ m BAS Sep-Stik ODS (Bioanalytical Systems)

Mobile phase: 50 mM pH 5.0 ammonium acetate buffer

Flow rate: 0.2

Injection volume: 0.5

Detector: UV 250

CHROMATOGRAM

Retention time: 0.5

Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

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: dialysate

Sample preparation: Inject an aliquot directly.

HPLC VARIABLES

Column: 100 × 1.5 μm ODS SepStik (Bioanalytical Systems)

Mobile phase: MeCN:50 mM pH 2.5 ammonium phosphate buffer 7:93

Flow rate: 0.1

Injection volume: 7

Detector: UV 250

CHROMATOGRAM

Retention time: 4.5

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

microbore; rat

REFERENCE

Steele, K.M.; Lunte, C.E. Microdialysis sampling coupled to on-line microbore liquid chromatography for pharmacokinetic studies. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 149–154

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 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: 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 \times 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 310

CHROMATOGRAM

Retention time: 4.25

Limit of detection: 1.9 $\mu\text{g}/\text{mL}$

OTHER SUBSTANCES

Simultaneous: caffeine (UV 298), chlorpheniramine (UV 265), guaifenesin (glycerylguaiacolate) (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 SilabatTM tablet using HPLC with diode array detection. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1555–1559

SAMPLE

Matrix: formulations

Sample preparation: 75 μL Sample + 6 mL mobile phase, vortex for 2 min, add 300 μL 300 $\mu\text{g}/\text{mL}$ acetaminophen, make up to 10 mL with mobile phase, mix for 2 min, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Spheri-5 ODS (Applied Biosystems)

Mobile phase: MeOH:buffer 30:70 (Buffer was 900 mL 50 mM Na_2HPO_4 + 18.75 mL tetrabutylammonium phosphate, pH adjusted to 6.8 with 1 N phosphoric acid.)

Flow rate: 1.2

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 3.8

Internal standard: acetaminophen

OTHER SUBSTANCES

Simultaneous: 5-aminosalicylic acid

KEY WORDS

acetaminophen is IS; rectal suspension; enema

REFERENCE

Henderson, L.M.; Johnson, C.E.; Berardi, R.R. Stability of mesalamine in rectal suspension diluted with distilled water. *Am.J.Hosp.Pharm.*, **1994**, *51*, 2955–2957

SAMPLE

Matrix: formulations

Sample preparation: Add one tablet to 10 mL MeOH and 80 mL dichloromethane, sonicate for 5 min, dilute to 100 mL with dichloromethane, dilute a 2 mL aliquot to 25 mL with mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Supelcosil

Column: 33 \times 4.6 3 μ m Supelcosil

Mobile phase: Dichloromethane:3.33% ammonium hydroxide in MeOH 98.5:1.5

Flow rate: 2

Injection volume: 10

Detector: UV 244

CHROMATOGRAM

Retention time: 4

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: propoxyphene

Noninterfering: impurities, p-nitrophenol, p-hydroxyacetophenone, 4-aminophenol

KEY WORDS

tablets; normal phase

REFERENCE

Asch, T.L.; Hunter, B.T. Simultaneous high-performance liquid chromatographic determination of propoxyphene and acetaminophen in pharmaceutical preparations. *J.Chromatogr.*, **1988**, *455*, 279–289

SAMPLE

Matrix: formulations

Sample preparation: Powder levodopa/carbidopa tablets or contents of capsules, weigh out an amount equivalent to about 100 ng levodopa, add 30 mL 0.1 M HCl, sonicate, make up to 50 mL with 0.1 M HCl, mix, filter (0.45 μ m), 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 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: 3% aqueous acetic acid

Flow rate: 1.5

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 9

Internal standard: acetaminophen

OTHER SUBSTANCES

Simultaneous: levodopa, carbidopa

KEY WORDS

tablets; capsules; acetaminophen is IS

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

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:buffer 20:80 (Buffer was 15 mM 1-butanefulfonic 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: 2.8

OTHER SUBSTANCES

Simultaneous: codeine, benzoic acid, 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: Dissolve capsules and tablets in MeOH:pH 4.0 water 1:1, shake for 1 (capsules) or 4 (tablets) h, dilute a 10 mL aliquot with 40 mL pH 3.2 water, filter (0.45 μm), collect last portion of filtrate, inject a 20 μL aliquot. (pH 3.2 and 4.0 water are prepared by adjusting pH of distilled water with phosphoric acid.)

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:buffer 7:93 (Buffer was 15 mM KH_2PO_4 + 2 mL triethylamine per liter. Adjusted to $\text{pH } 2.35 \pm 0.1$ with concentrated phosphoric acid.)

Column temperature: 40

Flow rate: 3

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Simultaneous: codeine, p-aminophenol, codeine-N-oxide, codeinone, degradation products

KEY WORDS

capsules; tablets; rugged

REFERENCE

Sisco, W.R.; Rittenhouse, C.T.; Everhart, L.A. Simultaneous high-performance liquid chromatographic stability-indicating analysis of acetaminophen and codeine phosphate in tablets and capsules. *J.Chromatogr.*, **1985**, *348*, 253-263

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: 8.5

Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES

Simultaneous: aspirin, p-aminophenol, 3-O-acetylascorbic acid, 2-O-acetylascorbic acid, Vitamin C, saccharin, 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 with water, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak C18

Mobile phase: MeOH:water:glacial acetic acid 45:55:2 containing 5 mM octanesulfonic acid

Flow rate: 2.5

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 1.5

OTHER SUBSTANCES

Simultaneous: guaifenesin, pseudoephedrine, pholcodine, methyl paraben, ethyl paraben, propyl paraben, butyl paraben

KEY WORDS

cough mixture

REFERENCE

Carnevale, L. Simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture by high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, *72*, 196-198

SAMPLE

Matrix: formulations

Sample preparation: Add 1 tablet to 95 mL water, place on a steam bath for 15 min, cool, mix for 15 min, sonicate, allow to stand, filter, inject a 13 μ L aliquot.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeOH:buffer 25:75 (Buffer was 0.01 N KH_2PO_4 + 50 mM KNO_3 , adjusted to pH 4.5 with 3 N phosphoric acid.)

Flow rate: 1.1

Injection volume: 13

Detector: UV 283

CHROMATOGRAM

Retention time: 5.9

OTHER SUBSTANCES

Simultaneous: hydrocodone, p-aminophenol, hydromorphone, codeine, p-chloroacetanilide

KEY WORDS

tablets; stability-indicating

REFERENCE

Wallo, W.E.; D'Adamo, A. Simultaneous assay of hydrocodone bitartrate and acetaminophen in a tablet formulation. *J.Pharm.Sci.*, **1982**, *71*, 1115-1118

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 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 μ Bondapak C18

Mobile phase: MeOH:water: ammonium formate buffer 45:54:1 (Prepare ammonium formate buffer by diluting 34 mL 28-30% ammonia with 30 mL water, add 30 mL 98% formic acid (Caution! Exothermic!). When cool dilute this mixture (pH 3.9) to 100 mL with water.)

Flow rate: 2

Injection volume: 15

Detector: UV 280

CHROMATOGRAM

Retention time: 4

Internal standard: o-dinitrobenzene (11)

OTHER SUBSTANCES

Simultaneous: guaifenesin, dextromethorphan, 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: microsomal incubations

Sample preparation: 250 μ L Microsomal incubation + 1 mL ice-cold ethyl acetate + 100 μ L 50 mM 11 β -hydroxytestosterone, mix, add 1.5 mL ethyl acetate, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μ L MeOH:water 20:80, inject an aliquot.

HPLC VARIABLES

Guard column: 5 μ m Supelco LC-18

Column: 150 \times 4.6 5 μ m Supelco LC-18

Mobile phase: Gradient. A was MeOH:water 30:70 adjusted to pH 4.5 with glacial acetic acid. B was MeCN:MeOH 10:90 adjusted to pH 4.5 with glacial acetic acid. A:B 87:13 for 34 min, to 50:50 over 10 min, return to initial conditions over 5 min.

Flow rate: 1.3

Detector: UV 240

CHROMATOGRAM

Retention time: 1.8

Internal standard: 11 β -hydroxytestosterone (26)

OTHER SUBSTANCES

Extracted: 6 β -hydroxytestosterone

REFERENCE

Chiba, M.; Nishime, J.A.; Lin, J.H. Potent and selective inactivation of human liver microsomal cytochrome P-450 isoforms by L-754,394, an investigational human immune deficiency virus protease inhibitor. *J.Pharmacol.Exp.Ther.*, **1995**, *275*, 1527-1534

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 8 μ m Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)

Mobile phase: MeCN:water 20:80

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: aspirin, phenacetin, salicylamide

REFERENCE

Jedrejewski, P.T.; Taylor, L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography. *J.Chromatogr.Sci.*, **1995**, *33*, 438–445

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 mobile phase 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: 4.75 (A), 3.68 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, mocllobemide, 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 2 μ L aliquot.

HPLC VARIABLES

Column: 30 \times 4.6 3 μ m Hypersil ODS

Mobile phase: MeOH:50 mM pH 6.2 phosphate buffer 15:85

Flow rate: 2

Injection volume: 2

Detector: UV 280

REFERENCE

Shah, K.P.; Chang, M.; Riley, C.M. Automated analytical systems for drug development studies. 3. Multivessel dissolution testing system based on microdialysis sampling. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1235–1241

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 8.3

OTHER SUBSTANCES

Simultaneous: aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranlycypromine, tripeleennamine

Interfering: N-acetylprocainamide

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 compounds in MeCN:water 80:20, inject a 1 μ L 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: 8

OTHER SUBSTANCES

Simultaneous: caffeine, dipyron (sulpyrin), guaifenesin (guaiacol glycerol ether), buccetin (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 semi-micro 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: 3.26

OTHER SUBSTANCES

Also analyzed: caffeine, dipyron (sulpyrin), buccetin (3-hydroxy-p-butyrophenetidine), phenacetin, mefenamic acid, aspirin, salicylamide, salicylic acid, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS

semi-micro

REFERENCE

Matsushima, Y.; Nagata, Y.; Niyomura, M.; Takakusagi, K.; Takai, N. Analysis of antipyretics by semi-micro liquid chromatography. *J.Chromatogr.*, **1985**, 332, 269–273

SAMPLE

Matrix: urine

Sample preparation: Dilute urine with water, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 5 μm Resolve C18 Guard-Pak (Waters)

Column: 100 × 8.5 μm Resolve C18 Radial Compression Module (Waters)

Mobile phase: MeOH:100 mM KH_2PO_4 :glacial acetic acid 4:95:1

Flow rate: 2.5

Injection volume: 20

Detector: UV 248

CHROMATOGRAM

Retention time: 4.47

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, acetaminophen glucuronide, acetaminophen sulfate

KEY WORDS

pharmacokinetics

REFERENCE

Goicoechea, A.G.; López de Alda, M.J.; Vila-Jato, J.L. A validated high-performance liquid chromatographic method for the determination of paracetamol and its major metabolites in urine. *J.Liq.Chromatogr.*, **1995**, *18*, 3257-3268

SAMPLE

Matrix: urine

Sample preparation: 0.3 mL Urine + 1 mL 100 mM pH 4.5 acetate buffer containing 20 mg/mL sodium pyrosulfite + 20 mg Limpet Acetone Powder. Incubate 3 h 37°, add 100 μL 400 mg/L phenacetin, extract twice with 4 mL of ether:dichloromethane:isopropanol 60:40:1. Add 0.5 mL pH 6.5 50 mM phosphate buffer containing 20 mg/mL sodium pyrosulfite, shake, allow phases to separate, evaporate upper organic layer under a stream of nitrogen and inject aqueous layer.

HPLC VARIABLES

Column: 100 \times 4.6 Chrompack microsphere C18

Mobile phase: Gradient. A was 50 mM pH 6.6 phosphate buffer. B was MeCN:50 mM pH 6.5 phosphate buffer 25:75. A:B from 90:10 to 10:90 over 50 min, maintain at 10:90 for 2 min.

Flow rate: 1.2

Injection volume: 250

Detector: UV 254

CHROMATOGRAM

Internal standard: phenacetin

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

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: urine

Sample preparation: 1 mL Urine + 4 mL 2 M pH 5.0 acetate buffer (+ 50 μ L β -glucuronidase-sulfatase at 37° overnight if required to hydrolyze conjugates). Centrifuge 200 μ L aliquots for 5 min, inject a 10-20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4.6 10 μ m μ Bondapak C18

Mobile phase: MeOH:HOAc:100 mM KH_2PO_4 7:0.75:92.25

Flow rate: 2

Injection volume: 10-20

Detector: UV 248; E, Bioanalytical Systems LC-4, TL-3 glassy carbon electrode + 0.60 V, Ag/AgCl reference electrode, output 10-50 nA/V

CHROMATOGRAM

Retention time: 8

Limit of detection: 70 ng/mL (UV), 15 ng/mL (E)

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Wilson, J.M.; Slattery, J.T.; Forte, A.J.; Nelson, S.D. Analysis of acetaminophen metabolites in urine by high-performance liquid chromatography with UV and amperometric detection. *J.Chromatogr.*, **1982**, 227, 453-462

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Akhtar, M.J.; Khan, S.; Hafiz, M. High-performance liquid chromatographic assay for the determination of paracetamol, pseudoephedrine hydrochloride and triprolidine hydrochloride. *J.Pharm. Biomed.Anal.*, **1994**, 12, 379-382 [simultaneous pseudoephedrine, triprolidine; formulations]

Bogusz, M.; Erkens, M. Reversed-phase high-performance liquid chromatographic database of retention indices and UV spectra of toxicologically relevant substances and its interlaboratory use. *J.Chromatogr.A*, **1994**, 674, 97-126 [gradient; also acebutolol, acecarbromal, acepromazine, acetazolamide, allobarbitol, allopurinol, alprazolam, alprenolol, amiloride, amiodarone, amitriptyline, amobarbital, amoxapine, amphetamine, aprobarbital, vitamin C, aspirin, atenolol, atrazine, atropine, barbital, benzocaine, benztropine, betaxolol, bisacodyl, brallobarbitol, bromazepam, brompheniramine, bupivacaine, bupranolol, buprenorphine, buspirone, butalbital, butaperazine, butobarbital, caffeine, carbamazepine, carbaryl, carbromal, chlordiazepoxide, chlormezanone, chloroquine, chlorpromazine, chlorprothixene, chlorthalidone, cimetidine, cinchocaine, clobazam, clomipramine, clonazepam, clonidine, clopamide, clopenthixol, clorazepate, clozapine, cocaine, codeine, colchicine, coumarin, cyclobarbitol, cyclopentobarbital, demoxepam, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diamorphine, diazepam, diazoxide, diclofenac, digoxin, diltiazem, dimenhydrinate, diphenhydramine, dipyrindamole, disopyramide, dothiepin, doxepin, doxylamine, droperidol, ephedrine, estazolam, ethacrynic acid, ethosuximide, fenfluramine, fenpropfen, fentanyl, flecainide, flumazenil, flunarizine, flunitrazepam, fluoxetine, flupenthixol, flurazepam, fluvoxamine, furosemide, glyburide, glipizide, glutethimide, guaifenesin, haloperidol, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxyzine, ibuprofen, imipramine, indomethacin, isoniazid, isosorbidedinitrate, ketamine, labetalol, methotrimeprazine, levorphanol, lidocaine, loperazolam, lorazepam, loxapine, mactrotiline, meclizine, medazepam, mephenytoin, mepivacaine, metapramine, methadone, methamphetamine, methaqualone, methohexital, methylphenidate, metipranolol, metoclopramide, metoprolol, metronidazole, midazolam, monolinuron, morazone, morphine, nadolol, nalorphine, naloxone, naproxene, nicotinamide, nicotine, niflumic acid, nikhethamide, nimodipine, nitrazepam, nitrendipine, nitrofurantoin, nitroglycerine, noscipine, orciprenaline, orphenadrine, oxazepam, oxazolam, oxprenolol, oxycodone, oxyphenbutazone, papaverine, pemoline, pentazocine, pentobarbital, pentoxifylline, perazine, perphenazine, meperidine, phenacetamide, phenacetin, phenazone, phencyclidine, phenel-

zine, pheniramine, phenobarbital, phentermine, phenylbutazone, phenytoin, pindolol, piroxicam, prazepam, primidone, probenecid, procainamide, procaine, prochlorperazine, promazine, promethazine, propafenone, propoxur, propranolol, propyphenazone, protriptyline, quinidine, quinine, ranitidine, reserpine, saccharin, salicylamide, salicylic acid, scopolamine, secobarbital, sotalol, spironolactone, strychnine, sulphadiazine, sulphiride, suprofen, temazepam, terfenadine, tetracaine, tetrazepam, thebaine, theobromine, theophylline, thiabendazole, thiopental, thioridazine, tiaprofenic acid, timolol, tocainide, tolbutamide, trancylpromine, trazodone, triamterene, triazolam, trichlormethiazide, trifluoperazine, trifluperidol, triflupromazine, trimethoprim, trimipramine, tripelenamine, tripolidine, verapamil, vinylbarbital, warfarin, yohimbine, zolpidem]

Gurley, B.J.; Zermatten, S.; Skelton, D. Determination of antipyrene in human serum by direct injection restricted access media liquid chromatography. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1591–1595 [extracted antipyrene; serum; column temp 37; acetaminophen is IS]

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs. *J.Anal.Toxicol.*, **1994**, *18*, 233–242 [also acepromazine, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrene, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, bebrisoquine, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloamphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenisn, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapam, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystiril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methylpoda, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital]

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tylprocainamide, aflatoxins, allylcyclopentylbarbituric acid, allylisobarbituric acid, alphaprodine, alphenal, aminoantipyrine, aminobenzamide, aminobenzoate, aminobenzoic, aminobenzoic acid, aminophylline, amitriptyline, amobarbital, amylocaine, anisic acid, anthranilamide, antipyrine, aprobarbital, aspirin, atropine, barbital, benzoate, benzocaine, benzoic acid, benzoyllecgonine, benzphetamine, brucine, butabarbital, butacaine, butethal, butyl paraben, caffeine, cannabichromene, cannabinal, carbostyryl, carvacrole, chloramphenicol, chlordiazepoxide, chloroethyltheophylline, chlorophenol, chloroquine, chlorotheophylline, chlorothiazide, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, cinchonidine, cinchonine, clonazepam, cocaine, codeine, colchicine, cortisone, coumarin, creatinine, cyclothiazide, cyheptamide, danazol, danthron, dapson, DES, despropionylfentanyl, dexamethasone, diallylbarbituric acid, diazepam, dibucaine, dichlorophene, dihydrate, diethylstilbestrol, diethyltryptamine, dihydroxyphenethyl alcohol, dihydroxyphenylacetic acid, dihydroxyphenylglycol, dimethylbarbituric acid, diphenhydramine, diphenoxylate, diphenylhydantoin, dipropyltryptamine, dithiodinicotinic acid, doxapram, dronabinol, dyphylline, estradiol, estriol, estrone, ethonitazene, ethosuximide, ethylmorphine, ethylnornicotine, ethylphenylmalonamide, ethyltolylmalonamide, etonitazene, eugenol, fenfluramine, fenopropfen, fentanyl, 5-fluorouracil, fluoxymesterone, flurazepam, furosemide, genticis, genticis acid, gitoxigenin, glutethimide, guaiacol, hexabarbital, hexahydrocannabinol, hexylresorcinol, hippuric acid, homovanillic acid, hydrocortisone, hydromorphone, hydroquinone, hydroxyethyltheophylline, hydroxyindoleacetic acid, hydroxyisoquinoline, hydroxymethyltestosterone, hydroxynicotinic acid, hydroxyphenobarbital, hydroxyphenylpyruvic acid, hydroxyquinoline, ibuprofen, imipramine, indoleacetic acid, indolecarboxaldehyde, indomethacin, isobutylmethylxanthine, isocarbostyryl, isoquinoline-N-oxide, lasix, levorphanol, lidocaine, LSD, meclizine, mefenamate, mefenamic, mefenamic acid, meperidine, mephenesin, mephobarbital, mepivacaine, mescaline, methocarbamol, methoxamine, methoxydichlorobenzoic acid, methyl salicylate, methyl dopa, methyl dopamine, methylparaben, methylphenidate, methylprimidone, methyltestosterone, methylxanthine, morphine, nalorphine, naloxone, naltrexol, naphthalene, naphthol, naphthoacetic acid, naproxen, nicotine, nicotinic acid, nikethamide, nitrofurantoin, nitrophenol, normethsuximide, oxazepam, oxyphenbutazone, papaverine, paraxanthine, pemoline, pentazocine, pentobarbital, phenacyclidine, phenetidine, phenobarbital, phentermine, phenylbutazone, phenytoin, physostigmine, piperonyl butoxide, prednisolone, prednisone, primidone, probenecid, procaine, progesterone, propiomazine, propyl paraben, pyrillamine, pyrithydione, pyrocatechol, quinoline-N-oxide, reserpine, resorcinol, saccharin, salicylamide, salicylate, salicylic, salicylic acid, secobarbital, stanozolol, sulfacetamide, sulfadimethoxine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, testosterone, tetracaine, tetrahydrocannabinol, thebaine, theobromine, theophylline, thiamylal, thienylcyclohexylpiperidine, thiobarbituric acid, thiobarbituric, thiosalicylate, thiosalicylic, thiosalicylic acid, tolbutamide, tolmetin, triamcinolone, trifluoromethylbenzoic acid]

Lurie, I.S.; McGuiness, K. The quantitation of heroin and selected basic impurities via reversed phase HPLC. II. The analysis of adulterated samples. *J.Liq.Chromatogr.*, **1987**, *10*, 2189–2204 [also impurities, acetaminophen, acetylcodeine, acetylmorphine, acetylprocaine, aminopyrene, amitriptyline, antipyrine, aspirin, barbital, benzotropine, caffeine, cocaine, codeine, diamorphine, diazepam, diphenhydramine, dipyrone, ephedrine, ethylmorphine, lidocaine, meconin, methamphetamine, methapyrilene, methaqualone, monoacetylmorphine, morphine, nalorphine, niacinamide, nicotinamide, noscapine, papaverine, phenacetin, phenmetrazine, phenobarbital, phenolphthalein, procaine, propanophenone, propoxyphene, pyrillamine, quinidine, quinine, salicylamide, salicylate, salicylic, salicylic acid, secobarbital, strychnine, tetracaine, thebaine, tripeleminamine, tropacocaine, vitamin B3, vitamin B5; electrochemical detection]

Wilson, T.D. Recent advances in HPLC analysis of analgesics. *J.Liq.Chromatogr.*, **1986**, *9*, 2309–2410 [also antipyrine, aspirin, buprenorphine, butorphanol, codeine, cyclazocine, diamorphine, dihydrocodeine, etorphine, fentanyl, heroin, hydrocodone, hydromorphone, ibuprofen, LAAM, levorphanol, meperidine, methadone, morphine, nalbuphine, naproxen, oxycodone, oxymorphone, pentazocine, phenacetin, phenazocine, phenylbutazone, propoxyphene, salicylamide, salicylic acid, zomepirac]

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- Mamolo, M.G.; Vio, L.; Maurich, V. High-pressure liquid chromatographic analysis of paracetamol, caffeine and acetylsalicylic acid in tablets. Salicylic acid quantitation. *Farmaco.[Prat.]*, **1985**, *40*, 111–123
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- Das Gupta, V.; Heble, A.R. Quantitation of acetaminophen, chlorpheniramine maleate, dextromethorphan hydrobromide, and phenylpropanolamine hydrochloride in combination using high-performance liquid chromatography. *J.Pharm.Sci.*, **1984**, *73*, 1553–1556

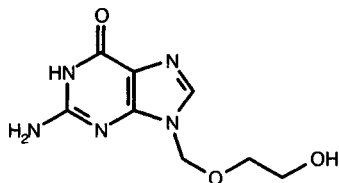
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- Quattrone, A.J.; Putnam, R.S. A single liquid-chromatographic procedure for therapeutic monitoring of theophylline, acetaminophen, or ethosuximide. *Clin.Chem.*, **1981**, *27*, 129–132
- West, J.C. Rapid HPLC analysis of paracetamol (acetaminophen) in blood and postmortem viscera. *J.Anal.Toxicol.*, **1981**, *5*, 118–121
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Acyclovir

Molecular formula: C₈H₁₁N₅O₃

Molecular weight: 225.2

CAS Registry No.: 59277-89-3, 69657-51-8 (sodium salt)



SAMPLE

Matrix: blood

Sample preparation: 100 µL Plasma + 10 µL 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: 18

Internal standard: acyclovir

OTHER SUBSTANCES

Extracted: cefitibuten

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; acyclovir is IS; column-switching

REFERENCE

Lim, J.M.; Kim, H.; Marco, A.; Mojaverian, P.; Lin, C.-C. Liquid chromatographic determination of cefitibuten, a new oral cephalosporin, in human plasma and urine. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 699-703

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg Sep-Pak Vac trifunctional C18 SPE cartridge with 1 mL MeOH and 1 mL buffer. 0.5 mL Plasma + 0.5 mL buffer, vortex, add to SPE cartridge, wash with 0.5 mL buffer, elute with 300 µL MeOH:5 mM sodium octanesulfonate 20:80 adjusted to pH 8.50 with 4 M NaOH, inject a 130 µL aliquot of the eluate. (Procedure was automated (ASPEC system). Buffer was 5 mM sodium octanesulfonate adjusted to pH 2.85 with concentrated orthophosphoric acid.)

HPLC VARIABLES

Guard column: 20 × 2 30-40 µm Perisorb RP-18 (change each day)

Column: 150 × 3.9 4 µm Nova Pak C18

Mobile phase: MeOH:10 mM Na₂HPO₄ + 10 mM sodium octanesulfonate 7:93 with the final apparent pH adjusted to 2.80 with concentrated orthophosphoric acid

Column temperature: 40

Flow rate: 1

Injection volume: 130

Detector: UV 250

CHROMATOGRAM

Retention time: 4

Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Swart, K.J.; Hundt, H.K.L.; Groenewald, A.M. Automated high-performance liquid chromatographic method for the determination of acyclovir in plasma. *J.Chromatogr.A*, **1994**, *663*, 65–69

SAMPLE

Matrix: blood

Sample preparation: Heat-inactivate serum at 56° for 1 h. 500 µL Serum + 50 µL 100 µg/mL guanosine, filter (Centrisart I M, 5000 cut-off), dilute ultrafiltrate 1:30 with mobile phase buffer, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4 5µm RP8e (Merck)

Mobile phase: MeOH: 100 mM pH 3.0 phosphate buffer containing 50 mM 1-octanesulfonic acid 5:95

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 6.36

Internal standard: guanosine (7.35)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Noninterfering: gangiclovir, zidovudine

KEY WORDS

serum; ultrafiltrate

REFERENCE

Nebinger, P.; Koel, M. Determination of acyclovir by ultrafiltration and high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *619*, 342–344

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 300 µL 3 M perchloric acid, vortex 15 s, centrifuge at 2000 g for 2 min, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 80 × 4 3 µm Nucleosil 120 3C18

Mobile phase: Gradient. A was 20 mM perchloric acid. B was MeCN:20 mM perchloric acid 45:55. A:B 100:0 for 3 min, 0:100 for 3 min (step gradient).

Column temperature: 30

Flow rate: 1.5

Injection volume: 20

Detector: F ex 260 em 375

CHROMATOGRAM**Retention time:** 2.3**Limit of detection:** 6-10 ng/mL

KEY WORDS

plasma

REFERENCE

Mascher, H.; Kikuta, C.; Metz, R.; Vergin, H. New, high-sensitivity high-performance liquid chromatographic method for the determination of acyclovir in human plasma, using fluorometric detection. *J. Chromatogr.*, **1992**, 583, 122-127

SAMPLE**Matrix:** blood**Sample preparation:** 200 μ L Blood + 600 μ L 16% trichloroacetic acid, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES**Guard column:** C18 Guard-Pak (Waters)**Column:** 100 \times 8 Nova-Pak C18 (in a Z module)**Mobile phase:** Gradient. A was 50 mM sodium hydrogen phosphate. B was MeOH:water 80:20 containing 5 mM NaH_2PO_4 . A:B 99:1 for 1.5 min, to 5:95 over 18.5 min, re-equilibrate at initial conditions for 10 min.**Flow rate:** 1.6**Detector:** UV 254

CHROMATOGRAM**Retention time:** 11.6**Limit of detection:** 250 ng/mL

OTHER SUBSTANCES**Extracted:** famciclovir (as penciclovir, the active metabolite)

KEY WORDS

mouse; pharmacokinetics

REFERENCE

Boyd, M.R.; Bacon, T.H.; Sutton, D. Antiherpesvirus activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine (BRL 39123) in animals. *Antimicrob. Agents Chemother.*, **1988**, 32, 358-363

SAMPLE**Matrix:** blood**Sample preparation:** Inject an aliquot directly onto the column.

HPLC VARIABLES**Guard column:** 20 \times 4.6 16 μ m Spheron Micro 300**Column:** 150 \times 3.2 12.5 μ m Spheron Micro 300 (glass column) (Lachema)**Mobile phase:** pH 1.8 Buffer containing 100 mM phosphoric acid and 100 mM sodium sulfate**Column temperature:** 45**Flow rate:** 1**Injection volume:** 10**Detector:** F ex 285 em 370

CHROMATOGRAM

Retention time: 2

Limit of detection: 100 ng/mL

KEY WORDS

plasma; effluent cooled to 2° before entering detector; GPC; dog; pharmacokinetics; direct injection

REFERENCE

Salamoun, J.; Sprta, V.; Sladek, T.; Smrz, M. Determination of acyclovir in plasma by column liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1987**, *420*, 197–202

SAMPLE

Matrix: blood, dialysate, urine

Sample preparation: 100 µL Plasma, urine, or dialysate + 100 µL 200 mM pH 7 phosphate buffer, 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

OTHER SUBSTANCES

Extracted: ceftibuten

KEY WORDS

plasma; acyclovir is IS

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, urine

Sample preparation: 1 mL Plasma or urine + 300 µL 3 M perchloric acid, vortex for 15 s, centrifuge at 2000 g for 2 min, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 80 × 4 3 µm Nucleosil 3C-18

Mobile phase: Gradient. MeCN:20 mM perchloric acid 0:100 for 3 min, 45:55 for 3 min (step gradient).

Column temperature: 30

Flow rate: 1.5

Injection volume: 20

Detector: F ex 260 em 375

CHROMATOGRAM

Limit of detection: 6-10 ng/mL (plasma); 25 ng/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Vergin, H.; Kikuta, C.; Mascher, H.; Metz, R. Pharmacokinetics and bioavailability of different formulations of aciclovir. *Arzneimittelforschung*, **1995**, *45*, 508–515

SAMPLE**Matrix:** blood, urine

Sample preparation: Plasma. 200 μ L Plasma + 200 μ L 200 mM pH 7.0 sodium phosphate, vortex, allow to sit for 15 min, add 800 μ L 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 \times 3.9 μ Bondapak 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**OTHER SUBSTANCES****Extracted:** cefibuten**KEY WORDS**

plasma; acyclovir is IS

REFERENCE

Kearns, G.L.; Reed, M.D.; Jacobs, R.F.; Ardite, M.; Yogev, R.D.; Blumer, J.L. Single-dose pharmacokinetics of cefibuten (SCH 39720) in infants and children. *Antimicrob.Agents Chemother.*, **1991**, *35*, 2078–2084

SAMPLE**Matrix:** perfusate**HPLC VARIABLES****Column:** Pecosphere 5C-C18**Mobile phase:** MeCN:0.1% acetic acid 2:98**Flow rate:** 1**Detector:** UV 254**REFERENCE**

Volpato, N.M.; Santi, P.; Colombo, P. Iontophoresis enhances the transport of acyclovir through nude mouse skin by electropulsion and electroosmosis. *Pharm.Res.*, **1995**, *12*, 1623–1627

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Shao, Z.; Park, G.-B.; Krishnomoorthy, R.; Mitra, A.K. The physicochemical properties, plasma enzymatic hydrolysis, and nasal absorption of acyclovir and its 2'-ester prodrugs. *Pharm.Res.*, **1994**, *11*, 237–242 [nasal perfusate]

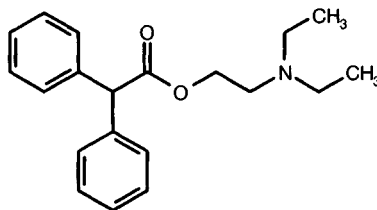
- Shao, Z.; Mitra, A.K. Bile salt-fatty acid mixed micelles as nasal absorption promoters. III. Effects on nasal transport and enzymatic degradation of acyclovir prodrugs. *Pharm.Res.*, **1994**, *11*, 243–240 [nasal perfusate]
- Macka, M.; Borák, J.; Seménková, L.; Popl, M.; Mikes, V. Determination of acyclovir in blood serum and plasma by micellar liquid chromatography with fluorimetric determination. *J.Liq.Chromatogr.*, **1993**, *16*, 2359–2386 [serum; plasma; fluorescence detection; LOD 80 ng/mL]
- Molokhia, A.M.; Niazy, E.M.; El-Hoofy, S.A.; El-Dardari, M.E. Improved liquid chromatographic method for acyclovir determination in plasma. *J.Liq.Chromatogr.*, **1990**, *13*, 981–989 [plasma; acetaminophen is IS]
- Cronqvist, J.; Nilsson-Ehle, I. Determination of acyclovir in human serum by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1988**, *11*, 2593–2601 [serum; non-interfering acetaminophen, allopurinol, baclofen, carbacholine, cefuroxime, chlorpropamide, cilastatin, cloxacillin, diazepam, dicumarol, digoxin, flucloxacillin, furosemide, fusidic acid, fusidic acid, glipizide, heparin, hydrochlorothiazide, imipenem, insulin, isoniazid, ketoprofen, metronidazole, naproxen, perphenazine, phenytoin, prednisolone, propranolol, pyrazinamide, pyridoxine, ranitidine, rifampicin, rifampin, spironolactone, streptomycin, sulfamethoxazole, trimethoprim, warfarin]
- Bouquet, S.; Regnier, B.; Quehen, S.; Brisson, A.M.; Courtois, P.; Fourtillan, J.B. Rapid determination of acyclovir in plasma by reversed phase high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1985**, *8*, 1663–1675 [plasma; acetaminophen is IS; LOD 250 ng/mL]
- Smith, R.L.; Walker, D.D. High-performance liquid chromatographic determination of acyclovir in serum. *J.Chromatogr.*, **1985**, *343*, 203–207 [serum; LOQ 1.2 µg/mL]
- Hoogewijs, G.; Massart, D.L. Development of a standardized analysis strategy for basic drugs, using ion-pair extraction and high-performance liquid chromatography. IV. Application to solid pharmaceutical dosage forms. *J.Liq.Chromatogr.*, **1983**, *6*, 2521–2541 [capsules; tablets; also benzocaine, caffeine, carbinoxamine, fenfluramine, flupentixol, lidocaine, melitracen, mepivacaine, phenylephrine, piperocaine, procaine, tetracaine; normal phase]
- Nilsson-Ehle, I. High-performance liquid chromatography for analyses of antibiotics in biological fluids. *J.Liq.Chromatogr.*, **1983**, *6*, Supp. 2, 251–293 [review]
- Zhang, C.; Dong, S.N. [Determination of acyclovir in human plasma by RP-HPLC]. *Yao.Hsueh.Hsueh.Pao.*, **1993**, *28*, 629–632
- Marini, D.; Pollino, G.; Balestrieri, F. Liquid chromatographic determination of acyclovir. *Boll.Chim.Farm.*, **1991**, *130*, 101–104

Adiphenine

Molecular formula: C₂₀H₂₅NO₂

Molecular weight: 311.4

CAS Registry No.: 64-95-9 (adiphenine), 50-42-0 (adiphenine hydrochloride)



HCl

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize brain with 4 volumes MeOH, stir for 15 min, centrifuge at 1300 g for 10 min, acidify supernatant with 300 μ L 1 M HCl, evaporate to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot. Plasma. Extract 1 mL plasma with 15 mL MeOH, stir for 15 min, centrifuge at 1300 g for 10 min, acidify supernatant with 300 μ L 1 M HCl, evaporate to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m Si 100 Lichrosorb

Mobile phase: Gradient. A was dichloromethane:diethylamine 100:0.2. B was dichloromethane:EtOH:diethylamine 80:20:0.2. A:B 100:0 for 4 min, to 50:50 over 13 min, maintain at 50:50 for 13 min.

Flow rate: 2

Detector: UV 254; Radioactivity

CHROMATOGRAM

Retention time: 3.7

KEY WORDS

normal phase; tritium labeled; ¹⁴C labeled; plasma; brain; rat

REFERENCE

Michelot, J.; Moreau, M.F.; Veyre, A.; Labarre, P.; Meyniel, G. Adiphenine plasma levels and blood-brain barrier crossing in the rat. *Eur.J Drug Metab. Pharmacokinet.*, **1985**, *10*, 273-278

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 melts, shake, filter (paper), inject a 25 μ L aliquot. Liquid formulations. Dilute 10 mL formulation to 100 mL with MeOH:0.5% acetic acid 1:1, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 μ Bondapak 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: 20

OTHER SUBSTANCES**Simultaneous:** dipyrone (metamizol), diphenhydramine, drofenine, ethyldiphenacetate**Interfering:** promazine**KEY WORDS**

tablets; suppositories; liquid formulations

REFERENCE

Facchini, G.; Zaccaro, 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**HPLC VARIABLES****Column:** 300 × 4 10 μm LiChrosorb RP-18**Mobile phase:** Gradient. A was 27 mM ammonia in water. B was 27 mM ammonia in MeOH. A:B from 100:0 to 70:30 over 6 min, maintain at 70:30 for 5 min, to 30:70 over 15 min, maintain at 30:70 for 15 min, to 0:100 over 2 min, maintain at 0:100 for 20 min.**Flow rate:** 2**Detector:** UV 254; Radioactivity**CHROMATOGRAM****Retention time:** 37.3**OTHER SUBSTANCES****Extracted:** metabolites**KEY WORDS**tritium labeled; ¹⁴C labeled**REFERENCE**

Michelot, J.; Madelmont, J.C.; Rousset, B.; Labarre, P.; Mornex, R.; Meyniel, G. Metabolism of adiphenine. II. Identification of major excretion metabolites in rats. *Xenobiotica*, **1982**, *12*, 457-462

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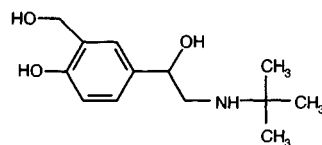
Michelot, J.; Moreau, M.F.; Madelmont, J.C.; Labarre, P.; Meyniel, G. Determination of adiphenine, diphenylacetic acid and diethylamino-ethanol by high-performance liquid chromatography. *J. Chromatogr.*, **1983**, *257*, 395-399 [same procedure as *Xenobiotica* 1982, 12, 457]

Albuterol

Molecular formula: C₁₃H₂₁NO₃

Molecular weight: 239.3

CAS Registry No.: 18559-94-9, 51022-70-9 (sulfate)



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)) KH₂PO₄ 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: 3.37

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, astemizole, benazepril, benperidol, benzocaine, benzoylegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzone, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephensesin, mephentermine, mepivacaine, metapramine, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam,

prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, reserpine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, suriclone, temazepam, tenoxicam, terfenadine, tetracaine, tetrazepam, thiopental, thiopropazine, thioridazine, tianeptine, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindsine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: aspirin, atenolol, chlormezanone, codeine, metformin, morphine, phenobarbital, phenol, ranitidine, ritodrine, sultopride, terbutaline, tiapride, toloxatone

KEY WORDS

whole blood; plasma

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 Sep-Pak SPE cartridge with 10 mL MeOH and 10 mL water. 500 μ L Serum + 1 mL water + 15 μ L 100 ng/mL fenoterol in water, mix, force slowly through SPE cartridge, wash twice with 2 mL water, elute with 2 mL MeOH (discard first 2 drops). Evaporate eluate to dryness under a stream of nitrogen at 40°, vortex for 1 min with 70 μ L buffer + 300 μ L 0.05% di(2-ethylhexyl) phosphate in ethyl acetate, centrifuge at 5000 g for 30 s, transfer organic phase to another tube and add 40 μ L buffer to it, vortex for 1 min, centrifuge at 5000 g for 30 s, transfer ethyl acetate layer to another tube and add 70 μ L 10 mM HCl to it, vortex 1 min, centrifuge at 5000 g for 30 s, remove acid layer and wash it with 150 μ L chloroform, centrifuge at 5000 g for 30 s, inject a 40-60 μ L aliquot of the aqueous phase. (Buffer was 70 mM NaH₂PO₄ + 1 mM chloride + 2 mM 1-heptanesulfonic acid, pH 6.8.)

HPLC VARIABLES

Column: 75 \times 4.6 3 μ m Ultrasphere ODS

Mobile phase: MeOH:buffer 25:75 (Buffer was 70 mM NaH₂PO₄ + 1 mM chloride + 2 mM 1-heptanesulfonic acid, pH 6.8.)

Flow rate: 0.5

Injection volume: 40-60

Detector: E, BioAnalytical System Model LC-4, TL-5 glassy carbon working electrode, Ag/AgCl reference electrode, +0.80 V, 10 nA full scale

CHROMATOGRAM

Retention time: 7

Internal standard: fenoterol (13)

Limit of detection: 0.4 ng/mL

OTHER SUBSTANCES

Noninterfering: buphenine, carbamazepine, dobutamine, epinephrine, ethosuximide, gentamycin, isoproterenol, isoxsuprine, metaproterenol, metaraminol, oxymetazoline, phenobarbital, phentolamine, phenylephrine, phenytoin, primidone, terbutaline, theophylline, valproic acid

KEY WORDS

serum; heparin interferes with IS; SPE; pharmacokinetics

REFERENCE

Tan, Y.K.; Soldin, S.J. Determination of salbutamol in human serum by reversed-phase high-performance liquid chromatography with amperometric detection. *J.Chromatogr.*, **1984**, *311*, 311-317

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 1 mL 100 mg (or 2.8 mL 500 mg) Extract-Clean silica SPE cartridge (Alltech) with 1 volume MeCN and 1 volume water. Add 1 mL urine or 3 mL plasma to the SPE cartridge, dry, wash with 1 volume water, wash with 1 volume MeCN, dry, elute with 2 volumes MeOH. Evaporate the eluate under reduced pressure at 45°, reconstitute with 1 (urine) or 0.3 (plasma) mL mobile phase, vortex for 1 min, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4 Chirex 3022 naphthyl urea (Phenomenex)

Column: 250 × 4 Chirex naphthyl urea (Phenomenex)

Mobile phase: Hexane:1,2-dichloromethane (sic):MeOH:trifluoroacetic acid 60.75:35:4.25:0.25

Flow rate: 1

Injection volume: 200

Detector: F ex 220 em 309

CHROMATOGRAM

Retention time: 22 (S-(+)), 27 (R-(-))

Limit of detection: 0.25 ng/mL

KEY WORDS

chiral; plasma; SPE; pharmacokinetics

REFERENCE

Boulton, D.W.; Fawcett, J.P. Determination of salbutamol enantiomers in human plasma and urine by chiral high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *672*, 103-109

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 100 mg Baxter C18 SPE cartridge with one volume MeOH and two volumes water. Dilute 10 µL urine to 500 µL with water. 500 µL Serum or diluted urine + 50 µL water, vortex for 30 s, add to SPE cartridge, wash with three 200 µL aliquots of water, elute with two 500 µL aliquots of MeOH. Evaporate the eluates to dryness under a stream of air at 40-45°, reconstitute the residue in 150 µL water, vortex for 30 s, centrifuge at 14000 g for 4 min, inject a 50 µL aliquot

HPLC VARIABLES

Guard column: 5 µm Adsorbosphere C-18

Column: 250 × 4.6 5 µm Adsorbosphere C-18

Mobile phase: MeCN:buffer 7:93 adjusted to pH 3.0 with 85% phosphoric acid (Buffer was 25 mM (NH₄)₂HPO₄ and 1 mM N,N-dimethyloctylamine.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 224; F ex 228 em 310

CHROMATOGRAM

Retention time: 7.1

Internal standard: albuterol

OTHER SUBSTANCES**Extracted:** atenolol

KEY WORDS

serum; SPE; albuterol is IS

REFERENCE

Chatterjee, D.J.; Li, W.Y.; Hurst, A.K.; Koda, R.T. High-performance liquid chromatographic method for determination of atenolol from human plasma and urine: Simultaneous fluorescence and ultraviolet detection. *J.Liq.Chromatogr.*, **1995**, *18*, 791–806

SAMPLE**Matrix:** bulk**Sample preparation:** Inject a 5 μ L aliquot of a solution.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m LiChrosphere Diol**Mobile phase:** Gradient. Carbon dioxide:MeOH containing 0.5% n-propylamine 70:30 for 9.5 min, to 55:45 over 12 min.**Column temperature:** 70**Flow rate:** 1.5**Injection volume:** 5**Detector:** UV

CHROMATOGRAM**Retention time:** 6.2**Limit of detection:** 1.3 μ g/mL

OTHER SUBSTANCES**Simultaneous:** impurities

KEY WORDS

300 bar; SFC

REFERENCE

Bernal, J.L.; del Nozal, M.J.; Rivera, J.M.; Serna, M.L.; Toribo, L. Separation of salbutamol and six related impurities by packed column supercritical fluid chromatography. *Chromatographia*, **1996**, *42*, 89–94

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute with water to a concentration of 833 μ g/mL, add a 300 μ L aliquot to 250 μ L 2 mg/mL mepivacaine hydrochloride in water, vortex, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 100 \times 8 4 μ m NovaPak C18 radial compression**Mobile phase:** Gradient. A was 2.5 mM PIC B-8 Low UV (Waters) in THF:water 40:60. B was water. C was MeOH:water 50:50. A:B:C 50:50:0 for 7.7 min, to 60:15:25 over 5.3 min, re-equilibrate at initial conditions for 5 min.**Flow rate:** 2**Injection volume:** 10**Detector:** UV 220

CHROMATOGRAM**Retention time:** 3.2**Internal standard:** mepivacaine (8.2)

OTHER SUBSTANCES

Simultaneous: fenoterol, ipratropium bromide, terbutaline

KEY WORDS

nebulizer solutions; stability-indicating

REFERENCE

Jacobson, G.A.; Peterson, G.M. High-performance liquid chromatographic assay for the simultaneous determination of ipratropium bromide, fenoterol, salbutamol and terbutaline in nebulizer solution. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 825–832

SAMPLE

Matrix: formulations

Sample preparation: Tablets, capsules. Mix tablets or capsules with 10 mL water, sonicate 30 min, centrifuge, inject an aliquot of the supernatant. Liquid formulations. Dilute liquid formulations with water, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4.5 μm LiChrospher 100 RP-18 endcapped

Mobile phase: MeOH:water 40:60 containing 2 mM KOH + 10 mM hexanoic acid

Flow rate: 0.4

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 3.5

Limit of detection: 1760 ng/mL

OTHER SUBSTANCES

Also analyzed: terbutaline, fenoterol

KEY WORDS

tablets; capsules; liquid formulations

REFERENCE

Ackermans, M.T.; Beckers, J.L.; Everaerts, F.M.; Seelen, I.G. Comparison of isotachopheresis, capillary zone electrophoresis and high-performance liquid chromatography for the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide in pharmaceutical dosage forms. *J.Chromatogr.*, **1992**, *590*, 341–353

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut silica (not C18) SPE cartridge twice with 1 mL MeOH and with 1 mL water. 1 mL Plasma + 200 μL 50 μg/mL bamethan sulfate in 1% potassium bicarbonate, add to SPE cartridge without applying vacuum, after 2 min apply vacuum to move samples through at 1 mL/min then increase vacuum to remove all liquid. Wash twice with 1 mL water and with 1 mL MeCN (draining completely each time). Elute with 1 mL MeOH, expelling last drop with positive pressure. Evaporate eluate under vacuum without heating, dissolve residue in 40 μL mobile phase, vortex, centrifuge at 300 g for 20 s, inject whole sample.

HPLC VARIABLES

Guard column: 15 × 3.2 Brownlee 7 μm C8

Column: 150 × 4.6 5 μm Ultrasphere Octylsilica

Mobile phase: MeCN:MeOH:water:phosphoric acid:KH₂PO₄:octanesulfonic acid 50:150:900:0.25:0.75:0.05 (v/v/v/v/w/w)

Flow rate: 1.6

Injection volume: 40

Detector: F ex 275 em 310

CHROMATOGRAM

Retention time: 7

Internal standard: bamethan sulfate (17.5)

Limit of quantitation: 0.2 ng/mL

KEY WORDS

SPE; plasma

REFERENCE

Gupta, R.N.; Fuller, H.D.; Dolovich, M.B. Optimization of a column liquid chromatographic procedure for the determination of plasma salbutamol concentrations. *J.Chromatogr.B*, **1994**, *654*, 205–211

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Si SPE cartridge by washing twice with 1 mL MeOH, twice with 1 mL water, and once with 1 mL 100 mM pH 9.2 K₂HPO₄. Add 1 mL plasma + 100 µL 500 ng/mL atenolol in water, wash twice with 1 mL water, centrifuge at 1000 g for 5 min, elute with 1 mL MeOH. Evaporate MeOH to dryness at 40° under a stream of air and dissolve residue in 200 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb S5 SCX

Mobile phase: MeOH:MeCN:water 40:40:20 containing 0.2% perchloric acid (apparent pH 1.7)

Flow rate: 1.5

Injection volume: 100

Detector: F ex 200 no emission filter

CHROMATOGRAM

Retention time: 8

Internal standard: atenolol (13)

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: terbutaline

Noninterfering: aminophylline, beclomethasone, cloprednol, dexamethasone, fenoterol, ipratropium bromide, methylprednisolone, orciprenaline, prednisolone, reproterol, rimiterol, salmeterol, sodium cromoglycate, theophylline

KEY WORDS

SPE; plasma

REFERENCE

McCarthy, P.T.; Atwal, S.; Sykes, A.P.; Ayres, J.G. Measurement of terbutaline and salbutamol in plasma by high performance liquid chromatography with fluorescence detection. *Biomed.Chromatogr.*, **1993**, *7*, 25–28

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond-Elut octadecylsilane SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer (do not allow to dry). 1 mL Serum + 10 μ L 100 μ g/mL bamethan in water + 2 mL buffer, mix, add to SPE cartridge, wash with 1 mL buffer, 2 mL water, 2 mL MeOH:MeCN 15:85. Dry under full vacuum for 10 min and elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at room temperature. Dissolve the residue in 200 μ L MeCN:triethylamine 199:1, heat at 45° for 20 min, add 10 μ L 5 mg/mL TAGIT in MeCN, heat at 45° for 2 h. Evaporate at room temperature under a stream of nitrogen, reconstitute in 250 μ L mobile phase, inject a 100 μ L aliquot. (Buffer was 100 mM Na₂HPO₄ adjusted to pH 7.3 with concentrated phosphoric acid. The chiral derivatizing agent TAGIT was 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl isothiocyanate. Prepare solutions of TAGIT in MeCN weekly.)

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m Brownlee octadecylsilyl

Mobile phase: MeCN:water 29:71 containing 0.1% triethylamine (pH adjusted to 4.0 with concentrated phosphoric acid)

Flow rate: 0.8

Injection volume: 100

Detector: F ex 223 no emission filter

CHROMATOGRAM

Retention time: 5.77 (R(-)), 6.83 (S(+))

Internal standard: bamethan (9, 10)

Limit of detection: 1 ng/mL

KEY WORDS

SPE; chiral; derivatization; serum

REFERENCE

He, L.; Stewart, J.T. A high performance liquid chromatographic method for the determination of albuterol enantiomers in human serum using solid phase extraction and chemical derivatization. *Bio-med.Chromatogr.*, **1992**, 6, 291-294

SAMPLE

Matrix: solutions

Sample preparation: Dilute 800 μ L solution to 10 mL with water, filter, inject a 20 μ L aliquot of the filtrate.

HPLC VARIABLES

Guard column: RP-18

Column: 125 \times 4 5 μ m LiChrosorb RP-18

Mobile phase: Gradient. MeCN:buffer 4:96 for 6 min, to 9:91 (step gradient). (Buffer was 40 mM NaH₂PO₄ 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 1979, 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

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 containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH

Column temperature: 17

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: k' 3.2 (first enantiomer)

KEY WORDS

chiral; α = 1.09

REFERENCE

Huynh, N.-H.; Karlsson, A.; Pettersson, C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglycyl-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: 5.57 (A), 3.27 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, 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, labelalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinamide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction also in paper

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 10 (Sumika Chemical Analysis Service)

Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:20:1

Flow rate: 1**Detector:** UV 230-280

CHROMATOGRAM**Retention time:** k' 5.41 (first enantiomer)

KEY WORDSchiral; $\alpha = 1.33$

REFERENCEOi, 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

HPLC VARIABLES**Column:** 250 \times 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:** 5-fluorouracil, acepromazine, acetaminophen, acetophenazine, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, bebrisoquine, benzocaine, benzoic acid, benzotroponine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbomal, 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, 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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, 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, mephenetermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, phenisuximide, phen-
 termine, phenylbutazone, phenylephrine, phenylpropranolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propyl-
 paraben, pseudoephedrine, puromycin, pyrillamine, pyrithyldione, quazepam, quinaldic
 acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albu-
 terol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sul-
 facetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine,
 sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, tema-
 zepam, testosterone, tetracaine, tetracycline, dronabinol, tetramisole, thebaine, theobro-
 mine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine,
 thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tran-
 ylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, tri-
 hexyphenidyl, trimethoprim, tripeleennamine, triproilidine, tropacocaine, tyramine, vera-
 pamil, 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 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: 1.76

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bame-
 thane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine,
 benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine,
 brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butetha-
 mate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorproma-
 zine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine,
 clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, de-
 sipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, die-
 thylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, di-
 phenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide,
 dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocris-
 tine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethoprop-
 azine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate,
 fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hys-
 cose, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine,
 laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophen-
 oxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine,
 mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdila-
 zene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, meth-

ylephedrine, 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, pimozone, 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, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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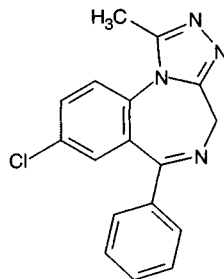
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Alprazolam

Molecular formula: C₁₇H₁₃ClN₄

Molecular weight: 308.8

CAS Registry No.: 28981-97-7



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)) KH₂PO₄ 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: 4.17

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cyproheptadine, cytarabine, dacarbazine, daunorubicin, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenpropofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephensesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methocarbamol, methotrexate, metipranolol, metoclopramide, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalor-

phine, naloxone, naltrexone, naproxen, nialamide, nicardipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thio-properazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclo-marol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, triflu-peridol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, videsine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: cycloguanil, debrisoquine, ketamine, lorazepam, methaqualone, metoprolol, nifedipine, piroxicam, sulindac

KEY WORDS

whole blood; plasma

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: 50 μL Serum + 25 μL 0.5 $\mu\text{g}/\text{mL}$ demoxepam in water + 100 μL 1 M pH 9.0 borate buffer, mix well, add 2 mL diethyl ether, vortex for 40 s, centrifuge at 1100 g for 5 min. Remove ether layer and evaporate it at 40° under nitrogen. Take up residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 2 μm Ultrasphere C18

Mobile phase: MeCN:MeOH:43 mM pH 2.4 sodium acetate buffer 8:45:47

Flow rate: 0.3

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 7

Internal standard: demoxepam (4)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, triazolam

Simultaneous: chlorpromazine, clonazepam, diazepam, flurazepam, hexobarbital, oxazepam, phenobarbital, temazepam

Noninterfering: amphetamine, buspirone, chlordiazepoxide, cocaine, cocathylene, flumazenil, midazolam, norcocaine

KEY WORDS

rat; serum

REFERENCE

Jin, L.; Lau, C.E. Determination of alprazolam and its major metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats. *J. Chromatogr. B*, **1994**, *654*, 77–83

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 $45 \times 4.12 \mu\text{m}$ TSK-gel G 3 PW (Tosohass); B $75 \times 4.6 \mu\text{m}$ Ultrasphere ODS C18 3**Mobile phase:** A 50 mM pH 7.5 phosphate buffer; B Gradient. X was MeCN. Y was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. X:Y 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:** 23

OTHER SUBSTANCES**Extracted:** bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethyloclobazam, 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

REFERENCELacroix, 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:** 3 mL Plasma + 30 μL 10 $\mu\text{g}/\text{mL}$ triazolam in water, mix 1 min, allow to stand for 15 min at room temperature, add to 3 mL Extrelut SPE cartridge and allow to soak in for 10 min, elute with 20 mL dichloromethane. Evaporate eluant at 30° under reduced pressure, take up residue in 1 mL MeCN:water 5:95, stand for 15 min, centrifuge at 14000 g for 2 min, remove supernatant. Inject a 250 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 7 min forward flush the contents of column A onto column B with mobile phase B. After 0.47 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B. When not in use, flush column A with mobile phase A. Between injections clean column A with two injections of 250 μL MeCN.

HPLC VARIABLES**Column:** A $30 \times 2.1 \times 10 \mu\text{m}$ MPLC cartridge PRP-1 (Kontron); B $100 \times 4.6 \mu\text{m}$ MPLC cartridge 5 μm RP-8 Spheri-5 (Kontron)**Mobile phase:** A 1 L water + 20 mL MeCN + 50 μL phosphoric acid (pH 3.2); B MeCN:buffer 40:60 (Buffer was 1 L water + 20 mL MeCN + 50 μL phosphoric acid (pH 3.2).)**Flow rate:** A 0.3; B 1

Injection volume: 250
Detector: UV 230

CHROMATOGRAM

Retention time: 7.6
Internal standard: triazolam (7.0)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, bromazepam, clobazam, diazepam, lorazepam, oxazepam

KEY WORDS

plasma; SPE; column-switching; pharmacokinetics

REFERENCE

Rieck, W.; Platt, D. High-performance liquid chromatographic method for the determination of alprazolam in plasma using the column-switching technique. *J.Chromatogr.*, **1992**, 578, 259–263

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 25 μ L 1 μ g/mL triazolam in toluene + 75 μ L 0.1% ammonium hydroxide, vortex 30 s, add 5 mL methylene chloride + 5 mL toluene, shake 15 min, centrifuge at 177 g for 10 min. Remove aqueous layer and freeze residual aqueous layer in dry ice-acetone for 30 s. Decant organic layer, dry under nitrogen at 50°, vortex residue with 200 μ L mobile phase, inject a 125 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m C18 (Supelco)
Mobile phase: MeOH:buffer 40:60 (Buffer was 1 mM phosphate and 3 mM hexyltriethylammonium phosphate in water at pH 7.4.)
Column temperature: 35
Flow rate: 2
Injection volume: 125
Detector: UV 221

CHROMATOGRAM

Retention time: 26
Internal standard: triazolam (30)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Schmith, V.D.; Cox, S.R.; Zemaitis, M.A.; Kroboth, P.D. New high-performance liquid chromatographic method for the determination of alprazolam and its metabolites in serum: instability of 4-hydroxy-alprazolam. *J.Chromatogr.*, **1991**, 568, 253–260

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 10 μ L 4 μ g/mL triazolam in methanol + 0.5 mL pH 9.12 saturated solution of sodium borate + 4.0 mL ethyl acetate:heptane 85:15, shake

10 min, centrifuge at 220 g for 10 min. Remove organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m C18 (IBM)

Mobile phase: MeCN:50 mM pH 6 potassium phosphate 30:70 (Between injections wash column with 10 mL MeCN:water 70:30 then 10 mL mobile phase.)

Flow rate: 1.5

Detector: UV 214

CHROMATOGRAM

Retention time: 12

Internal standard: triazolam (13.2)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: nitrazepam

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Miller, R.L.; DeVane, C.L. Alprazolam, α -hydroxy- and 4-hydroxyalprazolam analysis in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, 430, 180–186

SAMPLE

Matrix: blood

Sample preparation: 2 mL Serum or plasma + 100 μ L 1 μ g/mL IS in water + 0.5 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 $^{\circ}$, take up in 150 μ L mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 μ L aliquot of the lower layer.

HPLC VARIABLES

Column: 250 \times 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 water adjusted to pH 4.5 with orthophosphoric acid.)

Column temperature: 45

Flow rate: 2.5

Injection volume: 100

Detector: UV 202

CHROMATOGRAM

Retention time: 8.4

Internal standard: U-31485 (6.9)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, desipramine, protriptyline

Noninterfering: N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine

Interfering: imipramine, nortriptyline, triazolam

KEY WORDS

serum; plasma

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:** 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), 6.44 (B)**OTHER SUBSTANCES**

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azata-dine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlor-propamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomi-pramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclo-benzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, fu-rosemid, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, mida-zolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazo-line, 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, pro-methazine, propafenone, propantheline, propiomazine, propofol, propranolol, protripty-line, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinypra-zone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethyl-perazine, thiopental, thioridazine, thiothixene, timolol, tocinamide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, tri-methoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some details of plasma extraction in paper

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: Heat 5 mL urine + 1 mL temazepam in MeOH with 1 mL β -glucuronidase at 37° for 2.5 h, cool, adjust to pH 8.5 with saturated Na_2CO_3 , extract with 10 mL dichloromethane. Evaporate, take up the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Brownlee 5 μm RP-8

Mobile phase: MeCN:10 mM KH_2PO_4 :n-nonylamine 450:550:0.6 adjusted to pH 3.2 with phosphoric acid

Flow rate: 1.6

Detector: UV 225

CHROMATOGRAM

Retention time: 8

Internal standard: temazepam (7)

OTHER SUBSTANCES

Extracted: metabolites

Interfering: triazolam

REFERENCE

Fraser, A.D. Urinary screening for alprazolam, triazolam, and their metabolites with the EMIT d.a.u. benzodiazepine metabolite assay. *J.Anal.Toxicol.*, **1987**, 11, 263–266

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Hall, M.A.; Robinson, C.A.; Brissie, R.M. High-performance liquid chromatography of alprazolam in postmortem blood using solid-phase extraction. *J.Anal.Toxicol.*, **1995**, 19, 511–513 [SPE; blood]

Goldnik, A.; Gajewska, M. Determination of estazolam and alprazolam in serum by HPLC. *Acta Pol.Pharm.*, **1994**, 51, 311–312

Bogusz, M.; Erkens, M.; Franke, J.P.; Wijksbeek, J.; de Zeeuw, R.A. Interlaboratory applicability of a retention index library of drugs for screening by reversed phase HPLC in systematic toxicological analysis. *J.Liq.Chromatogr.*, **1993**, 16, 1341–1354 [gradient; also acebutolol, acetazolamide, aminophenazone, amphetamine, aspirin, atenolol, caffeine, carbomal, clomipramine, codeine, cyclobarbitol, diamorphine, diazepam, dibenzepine, diclofenac, droperidol, flunarizine, fluphenazine, flurazepam, hydrochlorothiazide, ibuprofen, lidocaine, lormetazepam, methadone, mianserin, mianserine, normethadone, oxycodone, perphenazine, phenacetin, phenazone, phenobarbital, phenylbutazone, promazine, propranolol, propyphenazone, salicylamide, strychnine, tetrazepam, theobromine, thio-pental, tilidine, tolbutamide, trifluoperazine, trifluoropromazine, triflupromazine, vinylbital, warfarin]

Atta-Politou, J.; Parissi-Poulou, M.; Dona, A.; Koutselinis, A. A simple and rapid reversed phase high performance liquid chromatographic method for quantification of alprazolam and α -hydroxyalprazolam in plasma. *J.Liq.Chromatogr.*, **1991**, 14, 3531–3546 [plasma; LOD 1 ng/mL; extracted hydroxyalprazolam; flunitrazepam (IS)]

Noggle, F.T., Jr.; Clark, C.R.; DeRuiter, J. Liquid chromatographic separation of some common benzodiazepines and their metabolites. *J.Liq.Chromatogr.*, **1990**, 13, 4005–4021 [also bromazepam, chlor-

diazepoxide, clobazam, clonazepam, clorazepate, demoxepam, diazepam, estazolam, fludiazepam, flunitrazepam, flurazepam, halazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam, triazolam]

Adams, W.J.; Bombardt, P.A.; Brewer, J.E. Normal-phase liquid chromatographic determination of alprazolam in human serum. *Anal.Chem.*, **1984**, *56*, 1590–1594

Alteplase

Molecular formula: C₂₇₃₆H₄₁₇₄N₉₁₄O₈₂₄S₄₅

Molecular weight: 59050.0

CAS Registry No.: 105857-23-6

SAMPLE

Matrix: formulations

Sample preparation: 100 μ L Formulation + 300 μ L 20 mM dithiothreitol in mobile phase, heat at 37° for 15 min, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 10 10 μ m TSK3000SW (Hewlett-Packard)

Mobile phase: 200 mM pH 6.8 NaH₂PO₄ containing 0.1% sodium dodecyl sulfate

Flow rate: 0.35

Injection volume: 25

Detector: UV 214

CHROMATOGRAM

Retention time: 16 (single-chain), 19 (two-chain)

KEY WORDS

injections; water; saline; 5% dextrose

REFERENCE

Lam, X.M.; Ward, C.A.; du Mée, C.P.R.C. Stability and activity of alteplase with injectable drugs commonly used in cardiac therapy. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1904–1909

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: streptokinase, urokinase, anistreplase

REFERENCE

Werner, R.G.; Bassarab, S.; Hoffmann, H.; Schlüter, M. Quality aspects of fibrinolytic agents based on biochemical characterization. *Arzneimittelforschung*, **1991**, *41*, 1196–1200

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 600 \times 7.5 Spherogel 3000 SW

Mobile phase: 230 mM pH 6.8 phosphate buffer containing 0.1% sodium dodecyl sulfate

Flow rate: 0.5

Detector: UV 280

CHROMATOGRAM

Retention time: 27

OTHER SUBSTANCES

Simultaneous: aggregates

Also analyzed: anistreplase, streptokinase, urokinase

KEY WORDS

SEC; GPC

REFERENCE

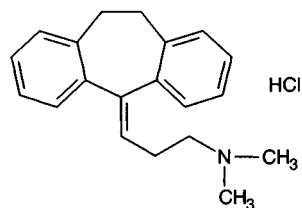
Werner, R.G.; Bassarab, S.; Hoffmann, H.; Schlüter, M. Quality aspects of fibrinolytic agents based on biochemical characterization. *Arzneimittelforschung*, **1991**, *41*, 1196–1200

Amitriptyline

Molecular formula: C₂₀H₂₃N

Molecular weight: 277.4

CAS Registry No.: 50-48-6 (amitriptyline), 549-18-8 (amitriptyline hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 500 μ L 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μ L 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 μ L 22 mM pH 2.5 KH₂PO₄/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 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 \times 4.6 Supelco C18

Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH₂PO₄ 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: blood

Sample preparation: 1 mL Plasma + 500 μ L 1 M sodium carbonate, vortex for 30 s, add 5 mL diethyl ether, vortex for 2 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μ L mobile phase, vortex for 30 s, centrifuge at 12000 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:water 62:38 adjusted to pH 3.5 with phosphoric acid

Column temperature: 40

Flow rate: 1.2

Detector: UV 262

CHROMATOGRAM

Retention time: 6.1

Internal standard: amitriptyline

OTHER SUBSTANCES

Extracted: pheniramine

Simultaneous: chlorpheniramine, diazepam, diltiazem, flurbiprofen, ibuprofen, itraconazole, ketoprofen, mebeverine, metoclopramide, phenylbutazone

KEY WORDS

dog; plasma; amitriptyline is IS

REFERENCE

El-Sayed, Y.M.; Niazy, E.M.; Khidir, S.H. High-performance liquid chromatographic method for the quantitative determination of pheniramine in plasma. *J.Liq.Chromatogr.*, **1995**, *18*, 763–777

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + doxepin + 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: 17.8

Internal standard: doxepin (11.6)

OTHER SUBSTANCES

Extracted: fluoxetine, norfluoxetine, nortriptyline

KEY WORDS

plasma

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

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)) KH₂PO₄ 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 240

CHROMATOGRAM

Retention time: 9.23

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

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

Interfering: etodolac, fluoxetine, nortriptyline, tiocloamarol

KEY WORDS

whole blood; plasma

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 + 500 μ L 250 μ g/mL protriptyline hydrochloride + 1 mL 500 mM NaOH + 4 mL toluene:n-hexane:isoamyl alcohol 77:22:3, mix for 10 min, centrifuge at 3000 rpm for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μ L MeOH, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.7 5 μ m Supelcosil LC-PCN cyanopropyl

Mobile phase: MeCN:MeOH:10 mM pH 7.2 potassium phosphate buffer 60:15:25 (Prepare buffer by mixing 194 mL 1.36 g/L KH_2PO_4 with 274 mL 1.74 g/L K_2HPO_4 .)

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 3.8

Internal standard: protriptyline (8.1)

OTHER SUBSTANCES

Extracted: norcyclobenzaprine, nortriptyline

Interfering: cyclobenzaprine

KEY WORDS

serum

REFERENCE

Wong, E.C.C.; Koenig, J.; Turk, J. Potential interference of cyclobenzaprine and norcyclobenzaprine with HPLC measurement of amitriptyline and nortriptyline: resolution by GC-MS analysis. *J. Anal. Toxicol.*, **1995**, *19*, 218–224

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 SPE cartridge, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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:** 5.6**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, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, 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:** acetazolamide, methadone, norfluoxetine, temazepam, trimipramine

KEY WORDS

SPE; plasma

REFERENCENichols, 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:** 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 μ L Serum + 50 μ L 5 μ g/mL trimipramine in 5% potassium bicarbonate + 700 μ L 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 μ L MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μ L aliquot of the elu

HPLC VARIABLES**Guard column:** 15 mm 7 μ m Brownlee RP-8**Column:** 150 \times 4.6 5 μ m 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:** 8.3**Internal standard:** trimipramine (9.6)**Limit of quantitation:** 5 ng/mL

OTHER SUBSTANCES

Extracted: clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, nortriptyline, protriptyline

Interfering: desmethyltrimipramine, maprotiline

KEY WORDS

SPE; serum

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: 500 μ L Serum + 250 μ L 100 mM lauryl sulfate, centrifuge at 2500 g for 8 min, inject a 250 μ L aliquot of the supernatant onto column A with mobile phase A, elute with mobile phase A for 6 min, backflush contents of column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B for 6 min and conduct analysis. When not in use flush column A with mobile phase A. Every eight injections backflush column A with MeCN:water 70:30.

HPLC VARIABLES

Column: A Guard-Pak 10 μ m Resolve CN (Waters); B 150 \times 3.7 μ m Separon SGX CN (Tessek)

Mobile phase: A MeCN:water 3:97; B MeCN:buffer 26:74 (Buffer was 50 mM phosphoric acid, 50 mM ammonium phosphate, and 28 mM diethylamine, pH 2.55.)

Flow rate: 1

Injection volume: 250

Detector: UV 210

CHROMATOGRAM

Retention time: 12

Limit of detection: 20–25 ng/mL

OTHER SUBSTANCES

Extracted: nortriptyline

KEY WORDS

column-switching; serum

REFERENCE

Dolezalová, M. On-line solid-phase extraction and high-performance liquid chromatographic determination of nortriptyline and amitriptyline in serum. *J.Chromatogr.*, **1992**, *579*, 291–297

SAMPLE

Matrix: blood

Sample preparation: For each 1 mL plasma or serum add 10 μ L 14 μ g/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 \times 4.6 10 μ m Hypersil MOS C8; B 20 \times 4.6 5 μ m Hypersil CPS CN + 250 \times 4.6 5 μ m 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: 5-10 ng/mL (1 ng/mL with 3 injections)

OTHER SUBSTANCES

Extracted: metabolites, desipramine, fluvoxamine, imipramine, maprotiline, nortriptyline

Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluoperidol

Interfering: clomipramine, 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: 1 mL Serum + 3 μ L 20 ng/mL clobazam in methanol + 1 mL saturated sodium borate (pH adjusted to 11 with 6 M NaOH) + 5 mL n-hexane, mix for 2 min, centrifuge at 3000 g for 10 min. Separate organic phase, evaporate to dryness under a stream of helium at 30°, reconstitute in 20 μ L mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 20 mm 40 μ m Pelliguard LC-8 (Supelco)

Column: 150 \times 4.6 Supelco 5 μ m C8

Mobile phase: MeCN:buffer 50:50 (Buffer was 10 mM NaH_2PO_4 + 1.2 mL/L butylamine, pH adjusted to 3 with phosphoric acid.)

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 9

Internal standard: clobazam (5)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: clomipramine, desipramine, imipramine, nortriptyline

Simultaneous: alprazolam, clonazepam, diazepam, flunitrazepam, haloperidol, lorazepam, maprotiline, nitrazepam, triazolam

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 + 20 μ L 10 μ g/mL clovoxamine + 120 μ L 2 M NaOH + 4 mL heptane:isopropanol 98:2, shake for 30 min, centrifuge at 3000 g for 10 min. Remove the organic layer and add it to 100 μ L 100 mM HCl, shake for 20 min, centrifuge at 3000 g for 10 min, inject a 20 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 120 \times 4.6 5 μ m Nucleosil C8

Mobile phase: MeCN:buffer 36:64 (Buffer was 16 mM KH_2PO_4 adjusted to pH 2.5 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 5.5

Internal standard: clovoxamine (3.3)

OTHER SUBSTANCES

Extracted: chlorimipramine, desipramine, doxepin, fluvoxamine, imipramine, nortriptyline, trimipramine

KEY WORDS

plasma

REFERENCE

Foglia, J.P.; Birder, L.A.; Perel, J.M. Determination of fluvoxamine in human plasma by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1989**, *495*, 295–302

SAMPLE

Matrix: blood

Sample preparation: Inject 200 μ L 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 \times 4 TSKprecolumn PW (Tosoh); B 150 \times 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: 17

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxapine, clomipramine, doxepin, desipramine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS

column-switching; use gradient to determine metabolites; serum

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 Plasma + 50 μ L MeOH:water 50:50 + 50 μ L 10 μ g/mL desmethyldoxepin in MeOH:water 50:50, mix, inject a 250 μ L aliquot of this mixture onto column A and elute to waste with mobile phase A. After 1.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 37-50 μ m 10 \times 1.5 Corasil RP C18; B 100 \times 4 Techsphere 3CN (HPLC Technology)

Mobile phase: A water; B MeCN:50 mM acetate buffer 60:40, pH 7

Flow rate: A 0.8; B 0.9

Injection volume: 250

Detector: UV 215

CHROMATOGRAM

Retention time: 12.5

Internal standard: desmethyldoxepin (8.5)

Limit of detection: 5-10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, nortriptyline

Simultaneous: cyanopramine, chloripramine, clomipramine, desipramine, doxepin, protriptyline, trimipramine

Noninterfering: tranlycypromine

Interfering: imipramine

KEY WORDS

column-switching; plasma

REFERENCE

Dadgar, D.; Power, A. Applications of column-switching technique in biopharmaceutical analysis. I. High-performance liquid chromatographic determination of amitriptyline and its metabolites in human plasma. *J.Chromatogr.*, **1987**, *416*, 99-109

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μ L Serum + 50 μ L 1 μ g/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 μ L 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 μ L mobile phase, inject a 40 μ L 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:** 9.1**Internal standard:** N-propionylprocainamide (6)**Limit of quantitation:** 25 ng/mL

OTHER SUBSTANCES**Extracted:** desipramine, 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, tobramycin, valproic acid, verapamil**Interfering:** thioridazine

KEY WORDS

serum; SPE

REFERENCELin, 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 240

CHROMATOGRAM**Retention time:** 7**Internal standard:** minaprine (5.5)**Limit of detection:** 20 ng/mL

OTHER SUBSTANCES**Simultaneous:** diltiazem, nortriptyline**Also analyzed:** amiodarone, clomipramine, desipramine, haloperidol, imipramine, propafenone, verapamil

KEY WORDS

serum

REFERENCEMazzi, 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:** 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 μ L 200 mM HCl, shake for 2 min, centrifuge. Inject a 20 μ L aliquot of the aqueous layer.

HPLC VARIABLES**Column:** 100 \times 3 octyl CP-tm-Spher C8 glass column (Chrompack)**Mobile phase:** MeCN:500 mM NaH₂PO₄ 35:65 adjusted to pH 2.2 with phosphoric acid**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 210

CHROMATOGRAM**Retention time:** 5.8**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Simultaneous:** doxepin, nortriptyline

KEY WORDS

serum

REFERENCEVan 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:** 500 μ L Plasma + 37 μ L 2 μ g/mL IS in MeOH + 500 μ L 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 μ L MeOH, inject a 50 μ L 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 \times 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:** desipramine, 2-hydroxydesipramine, 2-hydroxyimipramine, imipramine, metabolites, nortriptyline**Also analyzed:** doxepin, desmethyldoxepin, desmethylclomipramine, clomipramine, maprotiline, protriptyline**Noninterfering:** chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

KEY WORDS

plasma

REFERENCESutfin, 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:** Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then 200 μ L 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 μ L 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 $^{\circ}$ under compressed air. Reconstitute with 200 μ L mobile phase, vortex 10 s, inject 75-100 μ L aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25 μ L concentrated HCl.)

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Supelco**Mobile phase:** 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine (EtOH:MeCN:t-butylamine 98:2:0.05)**Flow rate:** 2**Injection volume:** 75-100**Detector:** UV 254

CHROMATOGRAM**Retention time:** 3.2**Internal standard:** promazine (5.2)**Limit of detection:** 2 ng/mL

OTHER SUBSTANCES**Simultaneous:** N-acetylprocainamide, amoxapine, amphetamine, buprion, chlordiaze-poxide, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, fluphenazine, 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, loxepin, maprotiline, methadone, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, trifluoperazine, trimeprazine, trimipramine, zimeldine**Noninterfering:** thiopropazine**Interfering:** chlorimipramine, doxepin, hydroxyamoxapine, meperidine, perphenazine, phenteramine, quinidine, thioridazine, trifluopromazine

KEY WORDS

normal phase; serum

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 μ L 1 μ g/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μ L 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 μ L 250 mM HCl, vortex for 30 s, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES**Guard column:** 50 \times 4.6 40 μ m C8 (Supelco)**Column:** 250 \times 4.6 5 μ m 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' 6.41**Internal standard:** loxapine (k' 7.18)**Limit of detection:** 2.5 ng/mL

OTHER SUBSTANCES

Extracted: chlordiazepoxide, chlorpromazine, desipramine, desmethyldiazepam, desmethylochlordiazepoxide, 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: Plasma. 1-5 mL Plasma + 1 mL 1 M NaOH, extract with mixed hexanes for 30 min, centrifuge. Remove a 9 mL aliquot of the hexane layer and evaporate it to dryness under a stream of nitrogen at 30°, dissolve residue in 100 μ L mobile phase, inject a 50 μ L aliquot. Whole blood. 10 mL Whole blood + 1 mL 1 M NaOH, extract with 15 mL mixed hexanes for 1 h. Remove an aliquot of the hexane layer and evaporate it to dryness, reconstitute the residue in 1 mL 100 mM HCl, extract with 5 mL chloroform by vortexing for 1 min, centrifuge. Remove a 4.5 mL aliquot of the chloroform layer, evaporate to dryness, dissolve in 10 μ L mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 10 μ m Micropak CN

Mobile phase: MeCN:20 mM ammonium acetate 90:10 (vary ammonium acetate concentration to achieve best separation)

Flow rate: 2.5

Injection volume: 10-50

Detector: UV 254; E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.8

Limit of detection: 10 ng/mL (UV); 0.1 ng/mL (E)

OTHER SUBSTANCES

Extracted: acetophenazine, bupropion, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, trifluorpromazine, 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: 1 mL Serum + 200 μ L 1.5 M NaOH, vortex for 5 s, add 6 mL hexane:isoamyl alcohol 99:1, add 200 μ L 1 μ g/mL perazine dimalonate in EtOH, rotate at 60 rpm for 30 min, centrifuge at 3000 g for 4 min. Remove the upper organic layer and add it to 20 μ L 2.5 mg/mL maprotiline in MeOH, vortex for 5 s, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 3.5 μ m Lichrosorb SI60

Mobile phase: MeCN:MeOH:ammonium hydroxide 250:55:13

Flow rate: 1.2

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 3.5

Internal standard: perazine (5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: N-acetylprocainamide, butaperazine, chlorimipramine, chlorpromazine, codeine, desipramine, dimethacrine, diphenhydramine, disopyramide, doxepin, hydroquinidine, maprotiline, melitracene, mesoridazine, nortriptyline, opipramol, perphenazine, procainamide, prochlorperazine, promazine, prothipendyl, protriptyline, quinidine, thioperazine, thioridazine, trifluoperazine

Noninterfering: acenocoumaron, acetaminophen, acetophenetidine, aspirin, benzodiazepines, bibenzepin, butriptyline, caffeine, chlorprothixene, clopenthixol, clothiapine, dixyrazine, droperidol, fluphenazine, haloperidol, hydroxyzine, isoniazid, methotrimeprazine,

metopimazine, moperone, noxiptyline, orphenadrine, pericyazine, phenprocoumon, pipothiazine, promethazine, salicylic acid, theophylline, thiopropazate, trimeprazine, trimipramine

Interfering: imipramine, pipamperone, thiethylperazine, thiothixene

KEY WORDS

maprotiline prevents adsorption on glass; serum; pharmacokinetics

REFERENCE

Edelbroek, P.M.; de Haas, E.J.M.; de Wolff, F.A. Liquid-chromatographic determination of amitriptyline and its metabolites in serum, with adsorption onto glass minimized. *Clin.Chem.*, **1982**, *28*, 2143-2148

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 200 μ L 10 μ g/mL protriptyline in water + 200 μ L 80 g/L NaHCO₃ + 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 μ L mobile phase, vortex for 15 s, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak 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: 3.29

Internal standard: protriptyline (12.20)

Limit of detection: 6 ng/mL

OTHER SUBSTANCES

Simultaneous: chlorpromazine, desipramine, desmethyldoxepin, disopyramide, doxepin, imipramine, maprotiline, nortriptyline, procainamide, propoxyphene, propranolol, thioridazine, trimipramine

Noninterfering: acetaminophen, caffeine, chlordiazepoxide, diazepam, methaqualone, salicylic acid, theophylline, 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: 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 μ L 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: μ Bondapak/Porasil

Column: μ Bondapak 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: 5.5

Internal standard: clomipramine (7.5)

Limit of detection: 0.7 ng

OTHER SUBSTANCES

Extracted: desipramine, imipramine, nortriptyline

Simultaneous: chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, diazepam, doxepin, flurazepam, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, secobarbital, thioridazine, trifluoperazine

Noninterfering: acetaminophen, codeine, meperidine

Interfering: propoxyphene

KEY WORDS

plasma

REFERENCE

Wong, S.H.Y.; McCauley, T. Reversed phase high-performance liquid chromatographic analysis of tricyclic antidepressants in plasma. *J.Liq.Chromatogr.*, **1981**, *4*, 849-862

SAMPLE

Matrix: blood, dialysate

Sample preparation: Adjust pH of serum samples to 7.4 and dialyze 3 mL serum against 5 mL dialysis buffer at 37° in PTFE chambers for 4 h. Inject 1 mL mobile phase, 1 mL water, 2 mL dialysate, 1 mL water, and 1 mL MeCN:water 50:50 onto column A. Elute column A onto column B with mobile phase for 30 s then remove it from the circuit. Elute column B with mobile phase and monitor the effluent. (Dialysis buffer was 3.998 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ + 0.775 g NaH_2PO_4 + 2.250 g NaCl + 0.055 g $\text{Hg}(\text{NO}_3)_2$ in 1 L water, pH was 7.4.)

HPLC VARIABLES

Column: A 10 × 6 packed with 40 μm material from a Bond Elut cartridge (cat. no. 620303); B 100 × 4 3 μm Spherisorb ODS Superpac

Mobile phase: MeCN:85% phosphoric acid:triethylamine:water 49.55:0.225:0.225:50

Flow rate: 0.65

Injection volume: 1000-2000

Detector: UV 238

CHROMATOGRAM

Retention time: 4.85

Limit of detection: 1 nM (5 mL sample)

OTHER SUBSTANCES

Extracted: nortriptyline

Simultaneous: alprazolam, chlorpromazine, chlorprothixene, clomipramine, desmethylmipramine, diazepam, flunitrazepam, fluphenazine, haloperidol, imipramine, maprotiline, perphenazine, promethazine, protriptyline, thioridazine, thioridazine sulfone, thioridazine sulfoxide, zimeldine, zuclopenthixol

Noninterfering: carbamazepine, clonazepam, lorazepam, nitrazepam, oxazepam, phenytoin

Interfering: desclomipramine, levomepromazine, trimipramine

KEY WORDScolumn-switching; serum

REFERENCE

Svensson, C.; Nyberg, G.; Mårtensson, E. High-performance liquid chromatographic quantitation of amitriptyline and nortriptyline in dialysate from plasma or serum using on-line solid-phase extraction. *J.Chromatogr.*, **1988**, *432*, 363–369

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μ L 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 μ L 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μ L 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 \times 4 30 μ m LiChrocart Aluspher RP-select B (Merck)

Column: 125 \times 4 5 μ m 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; UV 254

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Extracted: alprenolol, bromazepam, carbamazepine, chlordiazepoxide, 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, fenethylamine, fluoxetine, flupentixol, flurazepam, furosemide, glliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, lorazepam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyl dopa, 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, sulphamide, thioridazine, trazodone, triazolam, trimipramine, tripeleppamine, 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: 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 NaH₂PO₄:diethylamine 40:57.5:2.5

Flow rate: 2

Injection volume: 30

Detector: UV 220

CHROMATOGRAM

Retention time: 22.5

Internal standard: cianopramine (8.93)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: amoxapine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, dsipramine, 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, sulfuridazine, thioridazine, thiothixene, tranlycypromine, trazodone, trihexyphenidyl, triprolidine

Noninterfering: dextromethorphan, norphethidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: benztropine, promethazine, trimipramine

KEY WORDS

serum; liver

REFERENCE

McIntyre, I.M.; King, C.V.; Skafdis, 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: formulations

Sample preparation: Tablets, capsules. Crush tablet or capsule, weigh out amount corresponding to 2 mg amitriptyline, add 20 mL MeOH, shake for 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, inject a 10 µL aliquot. Liquid formulations. Take a 5 mL aliquot of a 2 mg/mL solution, make up to 10 mL with 1% HCl, remove a 5 mL aliquot and add it to 40 mL 1.25 mg/mL norephedrine.HCl in MeOH, make up to 100 mL with MeOH, inject a 10 µL aliquot.

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: 4
Internal standard: norephedrine (2.7)

OTHER SUBSTANCES

Also analyzed: chlorpromazine, imipramine, thioridazine, trifluoperazine

KEY WORDS

tablets; capsules; liquid formulations

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: 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 NaH₂PO₄ + 30 mL diethylamine.)

Flow rate: 2

Injection volume: 30

Detector: UV 214

CHROMATOGRAM

Retention time: 13

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: clomipramine, desipramine, dothiepin, doxepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS

there 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: 250 µL Microsomal incubation + 50 µL 1 M HCl, cool on ice, add desipramine, centrifuge at 16000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES**Column:** 300 × 3.9 μBondapak C18**Mobile phase:** MeCN:50 mM pH 6 potassium phosphate buffer 35:65**Flow rate:** 1.4**Detector:** UV 220

CHROMATOGRAM**Retention time:** 12**Internal standard:** desipramine (9)

OTHER SUBSTANCES**Extracted:** metabolites, nortriptyline**Noninterfering:** α-naphthoflavone, ketoconazole, quinidine

KEY WORDS

human; liver

REFERENCE

Schmider, J.; Greenblatt, D.J.; von Moltke, L.L.; Harmatz, J.S.; Shader, R.I. N-Demethylation of amitriptyline *in vitro*: Role of cytochrome P-450 3A (CYP3A) isoforms and effect of metabolic inhibitors. *J.Pharm.Exp.Ther.*, **1995**, *275*, 592–597

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:** 15.80 (A), 7.27 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordinazepoxide, 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, fenopropfen, fentanyl, flvoxate, 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, mephentoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclo-

bemide, 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, pimozone, 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, sulfapyrazone, 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

some 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 × 3.9 5 μm C8 Symmetry end-capped (prepared in the laboratory from Waters silica)

Mobile phase: MeOH:20 mM pH 7.00 potassium phosphate buffer 65:35

Column temperature: 23 ± 0.5

Flow rate: 1

Detector: UV

CHROMATOGRAM

Retention time: 22

OTHER SUBSTANCES

Simultaneous: propranolol

REFERENCE

O'Gara, J.E.; Alden, B.A.; Walter, T.H.; Petersen, J.S.; Niederländer, C.L.; Neue, U.D. Simple preparation of a C₈ HPLC stationary phase with an internal polar functional group. *Anal.Chem.*, **1995**, *67*, 3809–3813

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 Na₂HPO₄ 50:50

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.79

OTHER SUBSTANCES

Also analyzed: barbital, benzoic acid, 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: Prepare a 1 mg/mL solution in MeOH, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 2.75

OTHER SUBSTANCES

Simultaneous: benactyzine, buclizine, hydroxyzine, perphenazine, thioridazine, 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 \times 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, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin,

atenolol, atropine, avermectin, barbital, bebrisoquine, benzocaine, benzoic acid, benzotro-
pine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenor-
phine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbomal,
chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphe-
nesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine,
cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine,
colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide,
cymarin, danazol, danthron, dapsone, desipramine, dexamethasone, dextromethorphan,
dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbes-
trol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenor-
phine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epi-
nephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene,
etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, fenproporex,
fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone,
fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide,
glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone,
hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene,
imipramine, indomethacin, isocarbostryl, isocarboxazid, isoniazid, isoproterenol, isoxxsu-
prine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, loraze-
pam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid,
medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mepentermine, me-
phenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone,
methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol,
methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methyl-
phenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibole-
rone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefo-
pam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam,
norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone,
oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pento-
barbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-
metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phen-
termine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam,
prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylpar-
aben, pseudoephedrine, puromycin, pyrilamine, pyridylidone, quazepam, quinaldic acid,
quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol,
salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetam-
ide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfa-
methoxazole, 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, tranlycypromine, tri-
amcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, tri-
methoprim, tripeleminamine, 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 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 KH₂PO₄, 23.4:76.6 containing 0.05% nonylamine

Flow rate: 1.2

Detector: UV 214

CHROMATOGRAM**Retention time:** 16.5

OTHER SUBSTANCES**Simultaneous:** desipramine, desmethyldoxepin, doxepin, imipramine, loxapine, maprotiline, nortriptyline, trazodone

REFERENCE*Alltech Chromatography Catalog 300, 1993, p. 440*

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 Econosil C8**Mobile phase:** MeCN:buffer 30:70 (Buffer was 20 mM KH₂PO₄ and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)**Injection volume:** 20**Detector:** UV 210

CHROMATOGRAM**Retention time:** 11.0**Limit of quantitation:** < 1000 ng/mL

OTHER SUBSTANCES**Simultaneous:** amoxapine, carbamazepine, imipramine, nortriptyline**Also analyzed:** doxepin, desipramine, protriptyline, cyclobenzaprine, maprotiline

KEY WORDSUV spectra given

REFERENCERyan, 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**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' 2.54

REFERENCERoos, 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 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:** 3.74

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, dipyrizidamole, 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, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phen-tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piri-tramide, pizotifen, prazolol, pramoxine, prazosin, pronylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, 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, thiorida-zine, thiothixene, thonzylamine, timolol, tocainide, topropamine, tolycaine, tranlycy-promine, trazodone, trifluoperazine, trifluoperidol, 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: 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 \times 4.5 μm Lichrospher 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: 7.7

OTHER SUBSTANCES

Extracted: amphetamine, benzoylecgonine, cocaine (different gradient), codeine, diphenhydramine (different gradient), ephedrine (different gradient), lidocaine (different gradient), meperidine, morphine, nordiazepam, phenylpropanolamine (different gradient), norpropoxyphene, nortriptyline (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: 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 \times 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 \times 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 \times 3.2 5 μm C8 (Phenomenex) + 150 \times 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: 40 (B, C only)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210; UV 235

CHROMATOGRAM

Retention time: k' 4.3

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: amphetamine, benzoylecgonine, caffeine, codeine, cotinine, desipramine, diazepam, diphenhydramine, ephedrine, hydrocodone, hydromorphone, lidocaine, methadone, methamphetamine, morphine, nordiazepam, nortriptyline, oxazepam, pentazocine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, secobarbital

Interfering: flurazepam, imipramine

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: vitreous humor

Sample preparation: 600 μL Vitreous humor + 3 mL 0.1 M NaCl + 50 μL 4 $\mu\text{g}/\text{mL}$ desmethylclomipramine in water, mix for a few s, add to a C18 SepPak SPE cartridge 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 with 3 mL reagent by gravity. Elute with 3 mL MeOH and push air through to remove as much as possible. Evaporate under vacuum at 37°, vortex with 50 μL mobile phase for 1 min, inject a 25 μL aliquot. (Reagent was isopropanol:n-heptane:1 M sulfuric acid 40:320:1.)

HPLC VARIABLES

Guard column: 50 \times 4.6 30 μm Permaphase ETH (Du Pont)

Column: 250 \times 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: 23

Internal standard: desmethylclomipramine (26)

Limit of detection: 16.7 ng/mL

OTHER SUBSTANCES

Simultaneous: doxepin, imipramine, nortriptyline

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

KEY WORDS

SPE

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

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McGuire, M.; Wong, S.H.; Skrinska, V.; Fogelman, K.; Miles, W. Totally automated sequential analysis of tricyclic antidepressants by SPE module system and reversed-phase HPLC analysis with a base-deactivated C18 column. *J.Anal.Toxicol.*, **1996**, *20*, 65 [SPE]

Atta-Politou, J.; Tsarpalis, K.; Koutselinis, A. A modified simple and rapid reversed phase high performance liquid chromatographic method for quantification of amitriptyline and nortriptyline in plasma. *J.Liq.Chromatogr.*, **1994**, *17*, 3969–3982 [plasma; extracted metabolites, clomipramine, doxepin, nortriptyline, protriptyline; LOD 5 ng/mL; LOQ 10 ng/mL; simultaneous chlordiazepoxide, chlorpheniramine, chlorpromazine, phenobarbital, propranolol; non-interfering alprazolam, artane, bromazepam, diazepam, haloperidol, lorazepam, nitrazepam, oxazepam, pseudoephedrine, theophylline, triazolam; interfering propoxyphene]

Joron, S.; Robert, H. Simultaneous determination of antidepressant drugs and metabolites by HPLC. Design and validation of a simple and reliable analytical procedure. *Biomed.Chromatogr.*, **1994**, *8*, 158–164 [LOQ 3–17 ng/mL; plasma; also amitriptyline, amineptine, amoxapine, clomipramine, demoxipiline, desipramine, dosulepine, doxepine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, medifloxamine, mianserine, opipramol, quinupramine, tianeptine, toloxatone, trazodone, trimipramine, viloxazine]

Kirkland, J.J. Trends in HPLC column design for improved method development. *Am.Lab.*, **1994**, *26(9)*, 28K–28R [simultaneous desipramine, doxepin, trimipramine]

Kirkland, J.J.; Boyes, B.E.; DeStefano, J.J. Changing band spacing in reversed-phase HPLC. A technique for varying column stationary-phase selectivity. *Am.Lab.*, **1994**, *26(14) (Sept.)*, 36–41 [simultaneous desipramine, doxepin, trimipramine]

Oshima, N.; Kotaki, H.; Sawada, Y.; Iga, T. Tissue distribution of amitriptyline after repeated administration in rats. *Drug Metab.Dispos.*, **1994**, *22*, 21–25 [rat; plasma; liver; kidney; lung; brain; muscle; heart; extracted nortriptyline; clomipramine is IS; column temp 35; LOQ 10 ng/mL; pharmacokinetics]

Piperaki, S.; Parissi-Poulou, M.; Koupparis, M. A separation study of tricyclic antidepressant drugs by HPLC with β -cyclodextrin bonded stationary phase. *J.Liq.Chromatogr.*, **1993**, *16*, 3487–3508 [simultaneous chloripramine, doxepin, imipramine, maprotiline, nortriptyline, protriptyline]

Bogusz, M.; Erkens, M.; Maier, R.D.; Schröder, I. Applicability of reversed-phase base-deactivated columns for systematic toxicological analysis. *J.Liq.Chromatogr.*, **1992**, *15*, 127–150 [simultaneous bralobarbital, diphenhydramine, fluphenazine, imipramine, pentobarbital, salicylamide, secobarbital, thiopental, thioridazine]

Coudore, F.; Ardid, D.; Eschalié, A.; Lavarenne, J.; Fialip, J. High-performance liquid chromatographic determination of amitriptyline and its main metabolites using a silica column with reversed-phase eluent. Application in mice. *J.Chromatogr.*, **1992**, *584*, 249–255 [mouse+asma;brain;simultaneous metabolites, nortriptyline;imipramine (IS)]

Härter, S.; Wetzel, H.; Hiemke, C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography. *Clin.Chem.*, **1992**, *38*, 2082–2086 [column-switching; not validated for extraction from biological fluids; interfering clomipramine, doxepin, fluoxetine; simultaneous clozapine, demethylodoxepin, desipramine, desmethylclomipramine, desmethylmaprotiline, fluvoxamine, imipramine, maprotiline, norfluoxetine, nortriptyline, oxaprotiline]

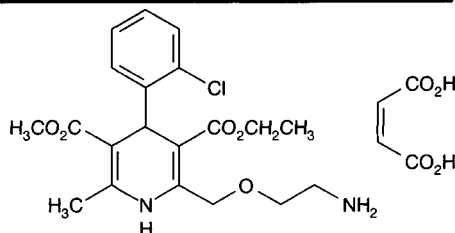
- Jalal, I.M.; Sa'sa', S.I.; Khalil, H.S. Simultaneous high performance liquid chromatographic determination of amitriptyline hydrochloride and perphenazine in tablet formulations. *J.Liq.Chromatogr.*, **1988**, *11*, 1531–1544 [tablets; simultaneous perphenazine; naphthalene (IS)]
- Liang, M.Z.; Huang, Y.; Qin, Y.P.; Zeng, J.Z. [Reversed phase HPLC determination of amitriptyline and nortriptyline in plasma]. *Hua.Hsi.I.Ko.Ta.Hsueh.Hsueh.Pao.*, **1987**, *18*, 144–147
- Kiel, J.S.; Abramson, R.K.; Smith, C.S.; Morgan, S.L. Development of a rapid extraction and high-performance liquid chromatographic separation for amitriptyline and six biological metabolites. *J.Chromatogr.*, **1986**, *383*, 119–127
- Smith, C.S.; Abramson, R.K.; Morgan, S.L. An investigation of the metabolism of amitriptyline using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1986**, *9*, 1727–1745 [rat; metabolites; tissue; liver]
- Terlinden, R.; Borbe, H.O. Determination of amitriptylinoxide and its major metabolites amitriptyline and nortriptyline in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *382*, 372–376 [plasma; column temp 45; extracted amitriptylinoxide; desipramine (IS); LOQ 10 ng/mL; pharmacokinetics; dog]
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Amlodipine

Molecular formula: C₂₀H₂₅ClN₂O₅

Molecular weight: 408.9

CAS Registry No.: 88150-42-9 (amlodipine), 111470-99-6 (amlodipine besylate), 88150-47-4 (amlodipine maleate)



SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 100 mg Bond Elut C2 SPE cartridge with 2 mL MeCN, 1 mL water, and 1 mL buffer. Add 500 μ L buffer, 50 μ L 200 ng/mL IS in MeOH:water 50:50, 1 mL plasma, and 500 μ L buffer sequentially to the SPE cartridge. Wash with 2 mL MeCN:water 20:80, wash with 1 mL MeCN, elute with 1 mL 2.5% ammonia in MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute with 50 μ L MeOH:100 mM pH 4.0 acetate buffer 50:50, inject an aliquot. (Buffer was 25 mM pH 7.0 phosphate buffer.)

HPLC VARIABLES

Column: 150 \times 2.1 Zorbax SB-Phenyl

Mobile phase: MeOH:100 mM pH 4.0 acetate buffer containing 2 mM sodium dodecyl sulfate and 1 mg/L EDTA

Column temperature: 30

Flow rate: 0.3

Detector: E, Antec Decade, Antec VT-03 analytical cell with 50 μ m spacer 0.95 V, ESA 5020 guard cell 0.5 V

CHROMATOGRAM

Retention time: 9

Internal standard: 2-[(2-aminoethoxy)methyl]-4-(2,3-dichlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester (UK52.829) (13)

Limit of quantitation: 0.2 ng/mL

KEY WORDS

SPE; narrow-bore; plasma

REFERENCE

Josefsson, M.; Zackrisson, A.-L.; Norlander, B. Sensitive high-performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single-step solid-phase sample preparation. *J. Chromatogr. B*, **1995**, 672, 310–313

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 2 μ g/mL desipramine in 10 mM HCl + 200 μ L 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 μ L 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 μ L mobile phase, inject a 20–40 μ L aliquot.

HPLC VARIABLES

Guard column: 45 \times 4.6 5 μ m Ultrasphere-ODS

Column: 250 \times 4.6 5 μ m 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: 10.6

Internal standard: desipramine (12.9)

Limit of quantitation: 2.5 ng/mL

KEY WORDS

plasma; rabbit; pharmacokinetics

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: Add IS to plasma, add pH 9 borate buffer, extract with diethyl ether. Remove the organic layer and add it to 100 μ L 100 mM citric acid, extract, inject an 80 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 \times 4.9 5 μ m Spherisorb nitrile (dog) or 125 \times 4.9 5 μ m Spherisorb ODS (rat)

Mobile phase: MeOH:buffer 35:65 (dog) or 45:65 (rat) (Buffer was 100 mM pH 5 N, N, N', N'-tetramethylethylenediamine phosphate.)

Flow rate: 1

Injection volume: 80

Detector: F ex 230 em 370

CHROMATOGRAM

Internal standard: 2-[(2-dimethylaminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine

KEY WORDS

plasma; dog; rat; pharmacokinetics

REFERENCE

Stopher, D.A.; Beresford, A.P.; Macrae, P.V.; Humphrey, M.J. The metabolism and pharmacokinetics of amlodipine in humans and animals. *J.Cardiovasc.Pharmacol.*, **1988**, *12 Suppl 7*, S55-S59

SAMPLE

Matrix: microsomal incubations, perfusate

Sample preparation: Basify 500 μ L perfusate or 1 mL microsomal incubation with 1 mL 200 mM pH 9.0 sodium borate buffer, extract into MTBE, evaporate extract to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 5 Hichrom HiRPB deactivated reverse-phase

Mobile phase: MeOH:25 mM pH 5.0 N, N, N', N'-tetramethylethylenediamine phosphate buffer 50:50

Flow rate: 1

Detector: UV 245

CHROMATOGRAM**Internal standard:** UK 46,129**Limit of detection:** 10 ng/mL

KEY WORDS

rat; liver

REFERENCE

Walker, D.K.; Humphrey, M.J.; Smith, D.A. Importance of metabolic stability and hepatic distribution to the pharmacokinetic profile of amlodipine. *Xenobiotica*, **1994**, *24*, 243–250

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 20 μ L aliquot of a 100 μ g/mL solution in MeOH or MeCN.

HPLC VARIABLES**Column:** 100 \times 4.6 PGC Hypercarb-S**Mobile phase:** MeOH:dichloromethane 75:25 containing 5 mM (1S)-(+)-10-camphorsulfonic acid**Column temperature:** 30**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 250

CHROMATOGRAM**Retention time:** 11 (S-(-)), 12 (R-(+))

OTHER SUBSTANCES**Also analyzed:** UK52.829

KEY WORDS

recirculate mobile phase for at least 12 h before use; chiral

REFERENCE

Josefsson, M.; Carlsson, M.; Norlander, B. Chiral ion-pair chromatographic separation of two dihydropyridines with camphorsulfonic acids on porous graphitic carbon. *J.Chromatogr.A*, **1994**, *684*, 23–27

SAMPLE**Matrix:** solutions**Sample preparation:** 100 μ L 0.1-1 mg/mL Amlodipine in MeCN:water 50:50 + 100 μ L 1 M pH 6.8 sodium borate buffer, vortex, add 50 μ L 100 mM (-)-(1R)-menthyl chloroformate in acetone, vortex, let stand for 5 min, add 1 mL water, add 2 mL dichloromethane, rotate for 10 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L MeCN, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 100 \times 10 Hypercarb-S porous graphitic carbon**Mobile phase:** MeCN:dichloromethane:formic acid 20:50:30**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 265

CHROMATOGRAM**Retention time:** 6.5, 7.5 (enantiomers)

OTHER SUBSTANCES**Simultaneous:** mexiletine, UK52.829**Interfering:** propranolol

KEY WORDSderivatization; chiral

REFERENCE

Josefsson, M.; Carlsson, B.; Norlander, B. Fast chromatographic separation of (-)-menthyl chloroformate derivatives of some chiral drugs, with special reference to amlodipine, on porous graphitic carbon. *Chromatographia*, **1993**, *37*, 129–132

SAMPLE**Matrix:** urine**Sample preparation:** Inject 1 mL urine.

HPLC VARIABLES**Column:** two 125 × 4.8 columns of Spherisorb 5 ODS in series**Mobile phase:** Gradient. A was 100 mM ammonium acetate in water. B was 100 mM ammonium acetate in MeOH. A:B 100:0 for 15 min, to 90:10 (step gradient), to 10:90 over 1 h.**Flow rate:** 1 for 15 min, then 0.8**Injection volume:** 1000**Detector:** MS, VG 12-250 quadrupole, thermospray. For the first 15 min the column effluent was diverted from the detector and the make-up flow to the detector was 1 mL/min. After 15 min the column effluent mixed with the make-up solvent pumped at 0.2 mL/min and the mixture flowed to the detector. The make-up solvent was 100 mM ammonium acetate in MeOH:water 50:50.

KEY WORDSrat

REFERENCE

Beresford, A.P.; Macrae, P.V.; Alker, D.; Kobylecki, R.J. Biotransformation of amlodipine. Identification and synthesis of metabolites found in rat, dog and human urine/confirmation of structures by gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. *Arzneimittelforschung*, **1989**, *39*, 201–209

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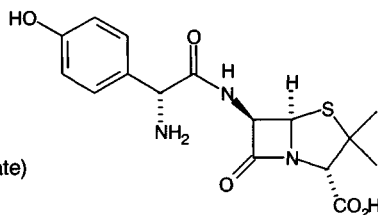
Shimooka, K.; Sawada, Y.; Tatematsu, H. Analysis of amlodipine in serum by a sensitive high-performance liquid chromatographic method with amperometric detection. *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1267–1272

Amoxicillin

Molecular formula: C₁₆H₁₉N₃O₅S

Molecular weight: 365.4

CAS Registry No.: 26787-78-0 (anhydrous), 61336-70-7 (trihydrate)



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 100 μ L 10 M urea, filter (Amicon MPS-1 with YMT membrane) while centrifuging at 1500 g for 30 min. 80 μ L Ultrafiltrate + 80 μ L 100 mM pH 9.0 borate buffer + 8 μ L 200 mM acetic anhydride in MeCN, let stand for 3 min, add 160 μ L reagent, heat at 60° for 10 min, inject a 20 μ L aliquot. (Reagent was 13.81 g 1,2,4-triazole in 60 mL water + 10 mL HgCl₂ solution (0.27 g HgCl₂ in 100 mL water), adjust pH to 9.0 \pm 0.05 with 4 M NaOH, dilute to 100 mL (Analyst 1985, 110, 1277).)

HPLC VARIABLES

Guard column: used but not specified

Column: 100 \times 3.5 μ m Chromospher Spherisorb ODS-2

Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM pH 4.6 sodium phosphate containing 10 mM thiosulfate.)

Flow rate: 0.8

Injection volume: 20

Detector: UV 328

CHROMATOGRAM

Limit of detection: 1000 ng/mL

KEY WORDS

derivatization; pharmacokinetics; ultrafiltrate; serum

REFERENCE

Huisman-de Boer, J.J.; van den Anker, J.N.; Vogel, M.; Goessens, W.H.F.; Schoemaker, R.C.; de Groot, R. Amoxicillin pharmacokinetics in preterm infants with gestational ages of less than 32 weeks. *Antimicrob. Agents Chemother.*, **1995**, *39*, 431–434

SAMPLE

Matrix: blood

Sample preparation: Condition an AASP C8 SPE cartridge (Varian) by washing with two 1 mL portions of MeCN and two 1 mL volumes of 67 mM pH 7.0 buffer. 150 μ L Plasma + 1.5 mL 67 mM pH 7.0 phosphate buffer + 20 μ L water, add a 1 mL aliquot to the SPE cartridge, wash with 200 μ L 67 mM pH 7.0 phosphate buffer, wash three times with 200 mg/L sodium azide in 67 mM pH 7.6 buffer. Place SPE cartridge on-line so that it is eluted onto the analytical column.

HPLC VARIABLES

Guard column: 4 \times 6 10 μ m μ Bondapak C18 Guard-Pak

Column: 250 \times 4.6 5 μ m Chromospher C18

Mobile phase: MeOH:buffer 20:80 (Buffer was 14.2 g NaH₂PO₄ in 900 mL water, add 12.5 mL 1 M tetrabutylammonium dihydrogenphosphate, adjust pH to 7.60 with dilute NaOH, make up to 1 L.) (100 \times 3 Chrompack reversed-phase saturation column between pump and injector used to saturate mobile phase with stationary phase.)

Column temperature: 30

Flow rate: 1
Detector: UV 234

CHROMATOGRAM

Retention time: 11.8
Limit of detection: 25 ng/mL

KEY WORDS

pharmacokinetics; plasma; SPE

REFERENCE

Krauwinkel, W.J.; Volkens-Kamermaans, N.J.; van Zijtveld, J. Determination of amoxicillin in human plasma by high-performance liquid chromatography and solid phase extraction. *J.Chromatogr.*, **1993**, *617*, 334-338

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 150 μ L 20% perchloric acid, centrifuge at 2000g for 4 min, inject a 20 μ L aliquot of the supernatant within 15 min.

HPLC VARIABLES

Column: 80 \times 4 Nucleosil 120 3C18
Mobile phase: MeCN:20 mM methanesulfonic acid 7.5:92.5
Injection volume: 20
Detector: E, ESA Model 5010, 0.78 V then F ex 255 em 400 (Oxidation by the electrochemical detector produces a fluorescent product which is then detected.)

CHROMATOGRAM

Retention time: 2.1
Limit of detection: 50 ng/mL

KEY WORDS

plasma

REFERENCE

Mascher, H.; Kikuta, C. Determination of amoxicillin in plasma by high-performance liquid chromatography with fluorescence detection after on-line oxidation. *J.Chromatogr.*, **1990**, *506*, 417-421

SAMPLE

Matrix: blood
Sample preparation: 500 μ L 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 0.5 mL 2 M NaOH, let stand for 5 min, add 0.5 mL 2 M HCl, add 2 mL 0.002% mercury(II) chloride in 500 mM Na₂HPO₄ (to adjust pH to 6.0), heat at 50° for 25 min, add 6 mL ethyl acetate saturated with water, 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 μ L MeOH containing IS, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 5 μ m Nucleosil C18
Mobile phase: MeOH:water 55:45
Column temperature: 55
Injection volume: 20
Detector: F ex 355 em 435

CHROMATOGRAM**Internal standard:** methyl anthranilate**Limit of detection:** 10 ng/mL

KEY WORDS

plasma; derivatization

REFERENCE

Miyazaki, K.; Ohtani, K.; Sunada, K.; Arita, T. Determination of ampicillin, amoxicillin, cephalexin, and cephadrine in plasma by high-performance liquid chromatography using fluorometric detection. *J.Chromatogr.*, **1983**, 276, 478-482

SAMPLE**Matrix:** blood, broncho-alveolar lavage fluid**Sample preparation:** 100 μ L Plasma or 500 μ L broncho-alveolar lavage fluid + 2 mL water + 1.5 mL 10% trichloroacetic acid, vortex for 30 s, centrifuge at 5000 rpm for 5 min. 1.5 mL Supernatant + 0.2 mL 2 M NaOH + 0.5 mL 0.1% (w/v) HgCl₂ in 67 mM pH 4.8 phosphate buffer (Sorensen), after 5 min bring to pH 6.2 with 1 mL 0.67 M Na₂HPO₄, keep at 40° for 25 min. Add 3 mL ethyl acetate, shake horizontally for 5 min, centrifuge at 3015 g for 5 min. Evaporate the organic phase to dryness under nitrogen; reconstitute in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4 Spherisorb 5 ODS**Mobile phase:** MeOH:5 mM 1-heptanesulfonic acid adjusted to pH 3.7 with acetic acid 60:40**Flow rate:** 1**Injection volume:** 20**Detector:** F ex 345 em 425

CHROMATOGRAM**Retention time:** 4.67**Internal standard:** amoxicillin**Limit of detection:** 50 ng/mL (plasma), 10 ng/mL (lavage)

OTHER SUBSTANCES**Extracted:** ampicillin

KEY WORDS

derivatization; amoxicillin is IS; plasma

REFERENCE

Rossee, M.T.; Bogaert, M.G.; Valcke, Y.J. High-performance liquid chromatographic assay of ampicillin in plasma and broncho-alveolar lavage fluid, using fluorescence detection. *Chromatographia*, **1989**, 27, 243-246

SAMPLE**Matrix:** blood, middle ear fluid**Sample preparation:** Condition a 2.8 mL 500 mg Bond Elut C18 SPE cartridge with 4 mL MeOH and 1 mL 50 mM pH 6.8 phosphate buffer. 200 μ L Plasma or 50 μ L middle ear fluid + 35 μ L 60 μ g/mL cefadroxil in MeOH:water 5:95 + 1 mL 50 mM pH 6.8 phosphate buffer, vortex, add to the SPE cartridge, wash with 1 mL 50 mM pH 6.8 phosphate buffer, wash with 1 mL buffer, dry under vacuum, elute with 1 mL MeOH:water 40:60. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 35 μ L MeOH:water 5:95, inject a 25 μ L aliquot.

HPLC VARIABLES**Guard column:** 10 × 2.5 μm MOS Hypersil C8**Column:** 150 × 2.5 μm MOS Hypersil C8**Mobile phase:** MeCN:5 mM phosphate buffer containing 5 mM tetrabutylammonium 6:94, adjusted to pH 6.5 (After 14 min wash column with MeCN:buffer 25:75 for 2 min, re-equilibrate for 4 min.)**Column temperature:** 40**Flow rate:** 0.35**Injection volume:** 25**Detector:** UV 210

CHROMATOGRAM**Retention time:** 6.45**Internal standard:** cefadroxil (12.30)**Limit of quantitation:** 125 ng/mL (plasma); 500 ng/mL (fluid)

KEY WORDSplasma; SPE; pharmacokinetics

REFERENCEYuan, Z.; Russlie, H.Q.; Canafax, D.M. Sensitive assay for measuring amoxicillin in human plasma and middle ear fluid using solid-phase extraction and reversed-phase high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *674*, 93–99

SAMPLE**Matrix:** blood, middle-ear effusion**Sample preparation:** 75 μL Plasma or middle-ear effusion + 50 μL 50 μg/mL hydroflumethiazide in water, mix, add 25 μL 10% perchloric acid, vortex, add 25 μL KCl solution. Mix, centrifuge, remove supernatant, add 25 μL pH 10.4 800 mM Na₂HPO₄ to the supernatant, inject a 6 μL aliquot. (Hydroflumethiazide solution prepared by dissolving hydroflumethiazide in a few drops MeOH and then diluting with water.)

HPLC VARIABLES**Guard column:** 20 × 3.2 Brownlee C8 precolumn**Column:** 150 × 4.6 5 μm Zorbax C8**Mobile phase:** MeOH:MeCN:10 mM NaH₂PO₄, 10:2:88**Column temperature:** 40**Flow rate:** 1.4**Injection volume:** 6**Detector:** UV 230

CHROMATOGRAM**Retention time:** 3.9**Internal standard:** hydroflumethiazide (6.4)**Limit of quantitation:** 500 ng/mL

KEY WORDSchinchilla; plasma

REFERENCEErdmann, G.R.; Walker, K.; Giebink, G.S.; Canafax, D.M. High performance liquid chromatographic analysis of amoxicillin in microliter volumes of chinchilla middle ear effusion and plasma. *J.Liq.Chromatogr.*, **1990**, *13*, 3339–3350

SAMPLE**Matrix:** blood, tissue

Sample preparation: Homogenize (Ultra-Turrax) 300 mg tissue at 4° for 45 s, centrifuge. 500 µL Serum or tissue homogenate supernatant + 500 µL 200 mM pH 7.0 ammonium acetate, vortex for 30 s, add 1 mL MeCN, mix for 15 s, centrifuge at 3000 rpm for 10 min. Add 3 mL dichloromethane to the supernatant, vortex for 30 s, centrifuge at 3000 rpm for 5 min, inject a 50 µL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 25 × 4 5 µm LiChrospher RP 18 E

Column: 125 × 4 5 µm LiChrospher RP 18 E

Mobile phase: MeCN:20 mM pH 6.8 Na₂HPO₄ 3:97

Flow rate: 1

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Limit of detection: 100 ng/mL (serum), 200 ng/mL (tissue)

KEY WORDS

fat; colon; serum

REFERENCE

Martin, C.; Mallet, M.-N.; Sastre, B.; Viviani, X.; Martin, A.; De Micco, P.; Gouin, F. Comparison of concentrations of clavulanic acid (200 and 400 milligrams) administered with amoxicillin (2,000 milligrams) in tissues of patients undergoing colorectal surgery. *Antimicrob. Agents Chemother.*, 1995, 39, 94-98

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 200 µL Serum + 100 µL 10 M urea, ultrafilter with Amicon YMT membrane at 1500 g for 10 min. 200 µL Ultrafiltrate + 200 µL 0.1 M pH 9 borate buffer + 20 µL 0.2 M acetic anhydride solution in MeCN. Stand at RT for 3 min, add 400 µL reagent, heat at 60° in a water bath for 10 min, cool, inject a 40-80 µL aliquot. Urine. Dilute urine 10 fold with water and filter through 0.45 µm acrylate copolymer membrane. 200 µL Filtrate + 200 µL 0.1 M pH 9 borate buffer + 20 µL 0.2 M acetic anhydride solution in MeCN. Stand at RT 3 min, add 400 µL reagent, heat at 60° in a water bath for 10 min, cool, inject a 20-80 µL aliquot. (Reagent was 13.81 g 1,2,4-triazole in 60 mL water + 10 mL HgCl₂ solution (0.27 g HgCl₂ in 100 mL water), adjust pH to 9.0 ± 0.05 with 4 M NaOH, dilute to 100 mL.)

HPLC VARIABLES

Column: 150 × 4.6 7 µm Zorbax ODS-7

Mobile phase: MeCN:20 mM NaH₂PO₄:20 mM sodium thiosulfate:MeCN 24:38:38

Flow rate: 1

Injection volume: 20-80

Detector: UV 328

CHROMATOGRAM

Retention time: 6

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, cyclacillin (ciclacillin)

KEY WORDS

serum; derivatization

REFERENCE

Haginaka, J.; Wakai, J. High-performance liquid chromatographic assay of ampicillin, amoxicillin and cefaclor in serum and urine using a pre-column reaction with 1,2,4-triazole and mercury(II) chloride. *Analyst*, **1985**, *110*, 1277-1281

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. Wash Amicon YMB filter membrane by stirring gently in 200 mL 100 mM pH 7.0 sodium phosphate buffer for 30 min, blot dry with filter paper. Dilute serum with an equal volume of 100 mM pH 7.0 sodium phosphate buffer, filter (Amicon YMB) while centrifuging at 5° at 1000 g for 15 min, inject a 25-50 µL aliquot. Urine. Dilute 10-fold with 100 mM pH 7.0 sodium phosphate buffer. Remove a 600 µL aliquot and add it to 200 µL buffer, inject a 25 µL aliquot.

HPLC VARIABLES

Guard column: CO:PEL ODS C18

Column: 250 × 4.6 µBondapak C18

Mobile phase: MeOH:buffer 6:94 (serum) or 4:96 (urine) (Buffer was 100 mM KH₂PO₄ adjusted to pH 3.2 with phosphoric acid.)

Flow rate: 2.5

Injection volume: 25-50

Detector: UV 227

CHROMATOGRAM

Retention time: 4 (serum), 7 (urine)

Limit of detection: 500 ng/mL

KEY WORDS

derivatization; ultrafiltrate; serum; pharmacokinetics

REFERENCE

Foulstone, M.; Reading, C. Assay of amoxicillin and clavulanic acid, the components of Augmentin, in biological fluids with high-performance liquid chromatography. *Antimicrob. Agents Chemother.*, **1982**, *22*, 753-762

SAMPLE

Matrix: broncho-alveolar lavage fluid

Sample preparation: Filter 1 mL broncho-alveolar lavage fluid (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 post-column reaction. The column effluent passed through a 10 m × 0.3 mm coil of PTFE tubing irradiated by a GL-10 10 W mercury lamp and flowed to the detector.

CHROMATOGRAM

Retention time: 17

Internal standard: amoxicillin

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: aspoxicillin

KEY WORDS

ultrafiltrate; post-column reaction; amoxicillin is IS

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

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 200 mg amoxicillin trihydrate in 8 mL 200 mM pH 11.0 phosphate buffer, add 10 mL 70 mg/L sulfamethazine in solvent, make up to 20 mL with solvent, inject an aliquot within 1 min. Dissolve 200 mg amoxicillin sodium salt in 8 mL solvent, add 10 mL 70 mg/L sulfamethazine in solvent, make up to 20 mL with solvent, inject an aliquot within 1 min. (Solvent was MeOH:200 mM pH 7.0 potassium phosphate buffer: water 5:5:90.)

HPLC VARIABLES

Guard column: 40 × 4.6 10 μm LiChrosorb RP-2

Column: 250 × 4.6 7 μm Zorbax C8

Mobile phase: Gradient. A is MeOH:200 mM pH 7.0 potassium phosphate buffer:water 5:5:90. B is MeOH:200 mM pH 7.0 potassium phosphate buffer:water 50:5:45. A:B 95:5 for 5 min, to 35:65 over 30 min, to 95:5 over 7.5 min.

Column temperature: 30

Flow rate: 1

Injection volume: 25

Detector: UV 274

CHROMATOGRAM

Retention time: 11

Internal standard: sulfamethazine (35)

OTHER SUBSTANCES

Simultaneous: impurities, amoxicillin dimer, amoxicillin trimer, amoxicillin piperazine-2,5-dione, amoxicilloates

REFERENCE

De Pourcq, P.; Hoebus, J.; Roets, E.; Hoogmartens, J.; Vanderhaeghe, H. Quantitative determination of amoxicillin and its decomposition products by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, 321, 441–449

SAMPLE

Matrix: formulations

Sample preparation: Capsules. Open five capsules, dissolve contents and shells in water, make up to 1 L with water, shake thoroughly, filter (0.2 μm) an aliquot, dilute the filtrate 10-20-fold, inject an aliquot. Oral suspensions. Dilute 200-400-fold, filter, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 2.5 μm Hypersil ODS

Mobile phase: MeOH:50 mM phosphate buffer 3:97, adjusted to pH 7.0 with orthophosphoric acid

Flow rate: 0.4
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 6.5

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

capsules; oral suspensions

REFERENCE

Shakoor, O.; Taylor, R.B.; Moody, R.R. Analysis of amoxicillin in capsules and oral suspensions by high-performance liquid chromatography. *Analyst*, **1995**, *120*, 2191–2194

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 μ m), inject a 10 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 200 \times 4.6 10 μ m 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: 2.555

Internal standard: albuterol sulfate (3.388)

OTHER SUBSTANCES

Simultaneous: dicloxacillin

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 amoxicillin and dicloxacillin mixtures in capsules. *Analyst*, **1992**, *117*, 981–984

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water and filter (reject first few mL of filtrate)

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeOH:buffer:water 15:1:84 (Buffer was 50 mL 200 mM KH_2PO_4 + 5.7 mL 200 mM NaOH made up to 200 mL, pH 6.)

Flow rate: 1

Injection volume: 20

Detector: UV 235

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Simultaneous: clavulanic acid

KEY WORDS

tablets; suspensions

REFERENCE

Abounassif, M.A.; Abdel-Moety, E.M.; Mohamed, M.E.; Gad-Kariem, R.A. Liquid chromatographic determination of amoxycillin and clavulanic acid in pharmaceutical preparations. *J.Pharm. Biomed.Anal.*, **1991**, 9, 731-735

SAMPLE

Matrix: formulations

Sample preparation: Dilute and filter

HPLC VARIABLES

Column: 150 × 3.9 5 μ m Nova Pak C18

Mobile phase: MeOH:50 mM pH 4.0 phosphate buffer 3:97

Flow rate: 0.8

Detector: UV 254

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Simultaneous: clavulanic acid

Noninterfering: degradation products, mannitol, saccharin

KEY WORDS

oral suspensions; stability-indicating

REFERENCE

Tu, Y.H.; Stiles, M.L.; Allen, L.V.J.; Olsen, K.M.; Barton, C.I.; Greenwood, R.B. Stability of amoxicillin trihydrate-potassium clavulanate in original containers and unit dose oral syringes. *Am.J. Hosp.Pharm.*, **1988**, 45, 1092-1099

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 μ L aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 70 mm long Co:Pell ODS

Column: 300 × 4.6 10 μ m Chromegabond C18 (E.S. Industries)

Mobile phase: MeCN:MeOH:10 mM KH₂PO₄ 19:11:70

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM**Retention time:** 3.6**Limit of detection:** 342 ng/mL

OTHER SUBSTANCES**Simultaneous:** ampicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V

KEY WORDStablets; capsules; oral suspensions; injections

REFERENCEBriguglio, 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:** Grind capsule to a fine powder. Weigh 500 mg amoxicillin, make up to 500 mL with water, sonicate ca. 30 min keeping temp below 30°, filter (0.45 μ m), inject a 50 μ L aliquot of the filtrate within 3 h.

HPLC VARIABLES**Column:** Two 150 \times 4.6 5 μ m Spherisorb-ODS columns in series**Mobile phase:** Gradient. A was buffer. B was MeOH:MeCN 75:25. A:B 100:0 for 5 min, to 60:40 over 5 min, maintain at 60:40 for 5 min, to 100:0 over 5 min. (Buffer was 10 mL 0.5 M K_2HPO_4 + 90 mL 0.5 M KH_2PO_4 made up to 1 L, pH ca. 5.9.)**Flow rate:** 1**Injection volume:** 50**Detector:** UV 220

CHROMATOGRAM**Retention time:** 16

OTHER SUBSTANCES**Simultaneous:** impurities, 6-aminopenicillanic acid, amoxicillin penicilloic acids, p-hydroxyphenylglycine

KEY WORDScapsules

REFERENCEFong, G.W.K.; Martin, D.T.; Johnson, R.N.; Kho, B.T. Determination of degradation products and impurities of amoxicillin capsules using ternary gradient elution high-performance liquid chromatography. *J.Chromatogr.*, **1984**, *298*, 459–472

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 μ m polyvinylidene difluoride). Inject 2 mL into an LC system (150 \times 4.6 5 μ m Supelcosil LC-18 \geq CN: 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 2 mL fraction at retention time for amoxicillin (11.5 min), add 100 μ L reagent, evaporate

to 1 mL, inject a 200 μ L aliquot. (Reagent was 10 mM phosphoric acid, 10 mM KH_2PO_4 , and 10 mM sodium decanesulfonate.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-18

Mobile phase: MeCN:buffer 33:67 (Buffer was 15 mM phosphoric acid and 7.5 mM sodium dodecyl sulfate.)

Flow rate: 1

Injection volume: 200

Detector: UV 214

CHROMATOGRAM

Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES

Also analyzed: ampicillin, cephalixin, 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. *JAOAC 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 + 1 mL 1 M oxalic acid, heat at 60° for 10 min, centrifuge for 10 min, remove the supernatant and add it to 20 mL water and 400 μ L tributylamine, shake well, add 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 μ L portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μ m), 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 \times 4.6 10 μ m 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; Charm II assay

CHROMATOGRAM

Retention time: 3.91

OTHER SUBSTANCES

Extracted: cefadroxil, ticarcillin

Simultaneous: ampicillin, ceftiofur, cephalixin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G

KEY WORDS

SPE

REFERENCE

Zomer, E.; Quintana, J.; Saul, S.; Chazem, S.E. LC-Receptograms: A method for identification and quantitation of β -lactams in milk by liquid chromatography with microbial receptor assay. *JAOAC Int.*, **1995**, *78*, 1165–1172

SAMPLE

Matrix: milk

Sample preparation: 500 μ L Milk + 500 μ L 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 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 220 \times 2.1 5 μ m Spheri-5 phenyl microbore (UV detection) or 220 \times 4.6 5 μ m Spheri-5 phenyl microbore (MS detection)

Mobile phase: MeCN:MeOH:triethylamine:85% phosphoric acid:water 15:5:0.4:0.4:79.2 containing 2 mM octanesulfonate and 2 mM dodecanesulfonate (UV) or isopropanol:acetic acid in 200 mM ammonium acetate:water 1.5:5:93.5 (MS)

Column temperature: 40

Flow rate: 0.2-0.45 (UV) or 0.8-1.2 (MS)

Injection volume: 10-100

Detector: UV 220; MS, Finnigan MAT 4800 quadrupole, thermospray, source 320°, vaporizer 120°, pulsed positive ion negative ion

CHROMATOGRAM

Retention time: 8.2 (UV), 4 (MS)

Limit of detection: 100 ng/mL (UV); 200 ng/mL (MS)

OTHER SUBSTANCES

Also analyzed: ampicillin, cloxacillin

KEY WORDS

ultrafiltrate

REFERENCE

Voyskner, 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: reaction mixtures

Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 4.6 5 μ m Microsorb C8

Column: 250 \times 4.6 5 μ m Microsorb C8

Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 18:82

Flow rate: 1

Injection volume: 20

Detector: UV 236

CHROMATOGRAM

Retention time: 6.2

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: 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: 3.5

OTHER SUBSTANCES

Simultaneous: ampicillin, cefaclor, cephalixin, cephadrine

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: 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 containing 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: 3.5

OTHER SUBSTANCES

Simultaneous: ampicillin, cephalirin

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: 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 μ L water and 3 mL ethyl ether, vortex gently for 30 s, centrifuge at 2000 rpm for 3 min, discard the organic layer. Add 200 μ L 20% trichloroacetic acid solution to the aqueous phase, vortex for 15 s, add 200 μ L 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 μ L mobile phase, vortex thoroughly, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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

SAMPLE

Matrix: urine

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH then 5 mL water. 1 mL Urine + 100 μ L 0.5 M tetrabutylammonium bromide in water, vortex 30 s, add to SPE cartridge, elute with 9 mL MeCN:buffer 3:97, make up to 10 mL with mobile phase, inject an aliquot. (Buffer was 100 mL 0.5 M disodium hydrogen orthophosphate + 350 mL water adjusted to pH 4.85 with 1 M citric acid then made up to 500 mL.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere C18

Mobile phase: MeCN:pH 7.1 100 mM disodium hydrogen phosphate:water 32.5:100:900

Flow rate: 1.2

Injection volume: 30

Detector: UV 229

CHROMATOGRAM

Retention time: 20

Limit of quantitation: 7.5 μ g/mL

OTHER SUBSTANCES

Simultaneous: metabolites, amoxicillin piperazine-2',5'-dione, amoxicilloic acid

KEY WORDS

SPE

REFERENCE

Chulavatnatol, S.; Charles, B.G. High-performance liquid chromatographic determination of amoxicillin in urine using solid-phase, ion-pair extraction and ultraviolet detection. *J.Chromatogr.*, **1993**, *615*, 91–96

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- Harik-Khan, R.; Moats, W.A. Identification and measurement of β -lactam antibiotic residues in milk: Integration of screening kits with liquid chromatography. *J.AOAC Int.*, **1995**, *78*, 978–986 [simultaneous ampicillin, ceftiofur, cephalixin, cloxacillin, penicillin G; milk; gradient]
- Chesa-Jiménez, J.; Peris, J.E.; Torres-Molina, F.; Granero, L. Low bioavailability of amoxicillin in rats as a consequence of presystemic degradation in the intestine. *Antimicrob.Agents Chemother.*, **1994**, *38*, 842–847 [tissue homogenate; rat]
- Moats, W.A. Determination of ampicillin and amoxicillin in milk with an automated liquid chromatographic cleanup. *J.AOAC Int.*, **1994**, *77*, 41–45 [milk; extracted ampicillin, cephalixin; gradient; LOQ 10 ppb]
- Snippe, N.; Van de Merbel, N.C.; Ruiter, F.P.M.; Steijer, O.M.; Lingeman, H.; Brinkman, U.A.T. Automated column liquid chromatographic determination of amoxicillin and cefadroxil in bovine serum and muscle tissue using on-line dialysis for sample preparation. *J.Chromatogr.B*, **1994**, *662*, 61–70 [serum; muscle; cow; post-column reaction; LOD 50 ng/mL; LOD 200 ng/g; SPE; extracted; cefadroxil; dialysis]
- Straub, R.; Linder, M.; Voyksner, R.D. Determination of β -lactam residues in milk using perfusive-particle liquid chromatography combined with ultrasonic nebulization electrospray mass spectrometry. *Anal.Chem.*, **1994**, *66*, 3651–3658 [milk; LC-MS; electrospray; extracted ampicillin, ceftiofur, cephalixin, cloxacillin, penicillin G; LOD 10 ppb]
- Tyczkowska, K.L.; Voyksner, R.D.; Straub, R.F.; Aronson, A.L. Simultaneous multiresidue analysis of β -lactam antibiotics in bovine milk by liquid chromatography with ultraviolet detection and confirmation by electrospray mass spectrometry. *J.AOAC Int.*, **1994**, *77*, 1122–1131 [milk; cow; LC-MS; electrospray; UV detection; LOD 10 ppb; extracted ampicillin, ceftiofur, cephalixin, cloxacillin, penicillin G; column temp 40]
- Kaniou, I.P.; Zachariadis, G.A.; Stratis, J.A. Separation and determination of five penicillins by reversed phase HPLC. *J.Liq.Chromatogr.*, **1993**, *16*, 2891–2897 [simultaneous ampicillin, cloxacillin, dicloxacillin, penicillin G; LOD 30–50 pb]
- Parker, C.E.; Perkins, J.R.; Tomer, K.B.; Shida, Y.; O'Hara, K. Nanoscale packed capillary liquid chromatography-electrospray ionization mass spectrometry: analysis of penicillins and cephalosporins. *J.Chromatogr.*, **1993**, *616*, 45–57 [capillary HPLC; electrospray; LC-MS; serum; also ampicillin, carbenicillin, cefalothin, cefazolin, cefmenoxime, cefmetazole, cefoperazone, cefotaxime, cefotiam, cefoxitin, cephalixin, cloxacillin, dicloxacillin, penicillin G, piperacillin, sulbenicillin]
- Straub, R.F.; Voyksner, R.D. Determination of penicillin G, ampicillin, amoxicillin, cloxacillin and cephalixin by high-performance liquid chromatography-electrospray mass spectrometry. *J.Chromatogr.*, **1993**, *647*, 167–181
- Hsu, M.C.; Hsu, P.W. High-performance liquid chromatographic method for potency determination of amoxicillin in commercial preparations and for stability studies. *Antimicrob.Agents Chemother.*, **1992**, *36*, 1276–1279
- Leroy, P.; Gavriloff, C.; Nicolas, A.; Archimbault, P.; Ambroggi, G. Comparative assay of amoxicillin by high-performance liquid chromatography and microbiological methods for pharmacokinetic studies in calves. *Int.J.Pharm.*, **1992**, *82*, 157–164
- Nelis, H.J.; Vandenbranden, J.; De Kruijff, A.; De Leenheer, A.P. Liquid chromatographic determination of amoxicillin concentrations in bovine plasma by using a tandem solid-phase extraction method. *Antimicrob.Agents Chemother.*, **1992**, *36*, 1859–1863
- Doadrio, A.L.; Sotelo, J. Determination of hydrolysis constants for amoxicillin by liquid chromatography. *An.R.Acad.Farm.*, **1989**, *55*, 203–212

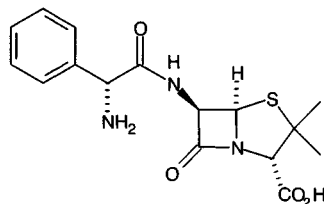
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- Martín, J.; Méndez, R.; Negro, A. Effect of temperature on HPLC separations of penicillins. *J.Liq.Chromatogr.*, **1988**, *11*, 1707–1716 [simultaneous ampicillin, cloxacillin, penicillin G, penicillin V, piperacillin; column temperature 15-55°]
- Haginaka, J.; Wakai, J. Liquid chromatographic determination of amoxicillin and its metabolites in human urine by postcolumn degradation with sodium hypochlorite. *J.Chromatogr.*, **1987**, *413*, 219–226 [extracted metabolites; post-column reaction; urine; LOD 1 µg/mL]
- Fong, G.W.K.; Johnson, R.N.; Kho, B.T. Study on the rate of epimerization of amoxicillin β-penicilloic acid to its α-form in aqueous solutions using high-performance liquid chromatography. *J.Chromatogr.*, **1983**, *255*, 199–207
- Nakagawa, T.; Shibukawa, A.; Uno, T. Liquid chromatography with crown ether-containing mobile phases. II. Retention behavior of β-lactam antibiotics in reversed- phase high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *239*, 695–706 [ampicillin, carbenicillin, cefradine, cephalixin, cephaloglycin, cephaloridin, ciclacillin, cloxacillin, dicloxacillin, oxacillin, penicillin G]
- Brooks, M.A.; Hackman, M.R.; Mazzo, D.J. Determination of amoxicillin by high-performance liquid chromatography with amperometric detection. *J.Chromatogr.*, **1981**, *210*, 531–535 [electrochemical detection; UV detection]

Ampicillin

Molecular formula: C₁₆H₁₉N₃O₄S

Molecular weight: 349.4

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)



SAMPLE

Matrix: bile, blood, urine

Sample preparation: Dilute bile and urine with 50 mM pH 7.0 phosphate buffer. 50 μ L Plasma + 100 μ L cephalixin in 50 mM pH 7.0 phosphate buffer, mix. Inject 100 μ L onto column A and elute to waste with mobile phase A, after 5 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 for 4 min before the next injection.

HPLC VARIABLES

Column: A 20 \times 3.9 25-40 μ m LiChrosorb RP-8; B 10 \times 4 Nova-Pak C8 guard column + 250 \times 4.6 5 μ m Ultracarb 5 ODS-30 (Phenomenex)

Mobile phase: A 50 mM pH 7.0 phosphate buffer; B Gradient. X was MeCN:20 mM pH 7.0 phosphate buffer 4:96. Y was MeCN:20 mM pH 7.0 phosphate buffer 30:70. X:Y 55:45 for 10 min, to 0:100 over 8 min, maintain at 0:100 for 12 min.

Flow rate: 1

Injection volume: 100

Detector: UV 230

CHROMATOGRAM

Retention time: 9.0

Internal standard: cephalixin (7.4)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: metampicillin

Noninterfering: acetaminophen, caffeine, ibuprofen, phenobarbital, sulbactam

KEY WORDS

column-switching; rat; pharmacokinetics; plasma

REFERENCE

Lee, H.; Lee, J.S.; Lee, H.S. Simultaneous determination of ampicillin and metampicillin in biological fluids using high-performance liquid chromatography with column switching. *J.Chromatogr.B*, **1995**, *664*, 335-340

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) for 10 min, centrifuge at 1000 g for 10 min, transfer supernatant to another tube, add 7 volumes dichloromethane, equilibrate for 10 min, shake by rotation (20 rpm) for 10 min, centrifuge at 1000 g for 10 min, inject aliquot of the upper aqueous layer. Urine. Centrifuge urine and dilute 1:20, inject an aliquot. Bile. Centrifuge bile and dilute 1:10, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid 8:92

Flow rate: 1

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 6.3

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Also analyzed: azlocillin, aztreonam, cefmenoxime, cefoperazone, cefotaxime, cefsulodin, 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: 500 μ L Serum + 1 mL 20% trichloroacetic acid, vortex for 15 s, centrifuge at 1000 g for 20 min. 1 mL Supernatant + 500 μ L 7% formaldehyde in 400 mM citric acid, vortex for 15 s, heat at 90° for 2 h, cool to room temperature. Either inject an aliquot of this solution directly or extract it twice with 3 mL portions of diethyl ether. Evaporate the extracts to dryness under reduced pressure, reconstitute with 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μ m RP-18 (Pierce)

Column: 100 \times 4.6 10 μ m RP-18 (Pierce)

Mobile phase: MeCN:100 mM pH 5.6 KH₂PO₄ 23:77

Flow rate: 1

Injection volume: 50

Detector: F ex 346 em 422

CHROMATOGRAM

Retention time: 7

Limit of detection: 2 ng/mL (with extraction)

KEY WORDS

serum; derivatization

REFERENCE

Lal, J.; Paliwal, J.K.; Grover, P.K.; Gupta, R.C. Determination of ampicillin in serum by high-performance liquid chromatography with precolumn derivatization. *J.Chromatogr.B*, **1994**, *655*, 142–146

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 μ g cefazolin, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 5 μm μBondapak C18

Mobile phase: MeOH:67 mM KH₂PO₄ 20:80

Flow rate: 1.5

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 9

Internal standard: cefazolin (14)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: bacampicillin (detected as ampicillin), lenampicillin (detected as ampicillin)

KEY WORDS

pharmacokinetics; 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

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 500 μL 2 M NaOH, let stand for 5 min, add 500 μL 2 M HCl, add 1 mL 0.1% mercury(II) chloride in buffer, let stand for 5 min, add 2 mL 0.67 M Na₂HPO₄ warmed to 40° to adjust pH to 6.2, heat mixture at 40° for 25 min, add 6 mL ethyl acetate saturated with water, 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 anthranilate (9 ?)

Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Interfering: cephalixin, cephradine

KEY WORDS

plasma; derivatization

REFERENCE

Miyazaki, K.; Ohtani, K.; Sunada, K.; Arita, T. Determination of ampicillin, amoxicillin, cephalixin, and cephradine in plasma by high-performance liquid chromatography using fluorometric detection. *J.Chromatogr.*, **1983**, *276*, 478–482

SAMPLE

Matrix: blood, broncho-alveolar lavage fluid

Sample preparation: 100 μ L Plasma or 500 μ L broncho-alveolar lavage fluid + 40 μ L 1 mg/mL (plasma) or 75 μ L 100 μ g/mL (broncho-alveolar lavage fluid) amoxicillin in 67 mM pH 4.8 phosphate buffer + 2 mL water + 1.5 mL 10% trichloroacetic acid, vortex for 30 s, centrifuge at 5000 rpm for 5 min. 1.5 mL Supernatant + 0.2 mL 2 M NaOH + 0.5 mL 0.1% (w/v) HgCl₂ in 67 mM pH 4.8 phosphate buffer (Sorensen), after 5 min bring to pH 6.2 with 1 mL 0.67 M Na₂HPO₄, keep at 40° for 25 min. Add 3 mL ethyl acetate, shake horizontally for 5 min, centrifuge at 3015 g for 5 min. Evaporate the organic phase to dryness under nitrogen; reconstitute in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4 Spherisorb 5 ODS

Mobile phase: MeOH:5 mM 1-heptanesulfonic acid adjusted to pH 3.7 with acetic acid 60:40

Flow rate: 1

Injection volume: 20

Detector: F ex 345 em 425

CHROMATOGRAM

Retention time: 3.45

Internal standard: amoxicillin (4.67)

Limit of detection: 50 ng/mL (plasma); 10 ng/mL (lavage)

KEY WORDS

derivatization; plasma

REFERENCE

Rosseel, M.T.; Bogaert, M.G.; Valcke, Y.J. High-performance liquid chromatographic assay of ampicillin in plasma and broncho-alveolar lavage fluid, using fluorescence detection. *Chromatographia*, **1989**, *27*, 243–246

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μ L Serum, plasma, or CSF + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 \times 2.1 40 μ m preparative grade C18 (Analytichem); B 250 \times 4.6 10 μ m Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH₂PO₄. 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: 4.98

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: acetazolamide

KEY WORDS

column-switching; serum; plasma

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, saliva, urine

Sample preparation: Plasma, saliva. 200 μ L Plasma or saliva + 10 μ L 60% perchloric acid + 200 μ L dichloromethane, vortex, centrifuge, inject a 50 μ L aliquot of the aqueous phase. Urine. 10 μ L Urine + 500 μ L 330 mM perchloric acid, mix, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4.6 Magnusphere C18 (Magnus Scientific, Sandbach, England)

Mobile phase: MeOH:67 mM pH 4.6 KH_2PO_4 15:85

Flow rate: 1

Injection volume: 50-100

Detector: UV 225

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Rogers, H.J.; Bradbrook, I.D.; Morrison, P.J.; Spector, R.G.; Cox, D.A.; Lees, L.J. Pharmacokinetics and bioavailability of sultamicillin estimated by high performance liquid chromatography. *J.Antimicrob.Chemother.*, **1983**, *11*, 435–445

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μ L Plasma + 200 μ L water + 100 μ L 70% perchloric acid: pH 5.4 buffer 25:75, vortex for 1 min, centrifuge at ca. 2400 g for 10 min. Remove 300 μ L supernatant and add it to 75 μ L 1 M NaOH, mix, inject a 100 μ L aliquot onto column A and elute to waste with mobile phase A, after 2 min collect the effluent from column A in a 1 mL sample loop, after 1 min inject the contents of the sample loop onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent from column B. Urine. Dilute urine if necessary. 80 μ L Urine + 720 μ L pH 4.85 buffer, vortex, inject a 20 μ L aliquot onto column A and elute to waste with mobile phase A, after 2 min collect the effluent from column A in a 1 mL sample loop, after 1 min inject the contents of the sample loop onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent from column B. (Prepare pH 5.4 buffer by dissolving 19.9 g Na_2HPO_4 and 8.4 g citric acid monohydrate in 250 mL water. Prepare pH 4.85 buffer by mixing 10 mL 500 mM Na_2HPO_4 and 350 mL water, adjust pH to 4.85 with 1 M citric acid, make up to 500 mL with water.)

HPLC VARIABLES

Column: A 15 \times 3.2 7 μ m New Guard RP18 + 3 \times 3 3 μ m Perkin-Elmer; B 100 \times 4.6 3 μ m Microspher C18 (Chrompack)

Mobile phase: A MeOH:buffer 17:83 containing 1 mM sodium hexylsulfate (Buffer was pH 7.4 phosphate buffer, ionic strength 0.05.); B MeOH:buffer 35:65 (plasma) or 30:70 (urine) (Buffer was pH 7.4 phosphate buffer, ionic strength 0.05.)

Flow rate: 1

Injection volume: 20-100

Detector: F ex 372 em 470 following post-column reaction. The effluent from column B mixed with 160 $\mu\text{g/mL}$ fluorescamine in MeCN pumped at 0.2 mL/min and the mixture flowed through a 5 m \times 0.4 mm i.d. knitted PTFE tube to the detector.

CHROMATOGRAM

Retention time: 8

Limit of detection: 570 nM (urine); 14 nM (plasma)

KEY WORDS

derivatization; plasma; post-column reaction; column-switching; heart-cut

REFERENCE

Lanbeck-Vallén, K. Carlqvist, J.; Nordgren, T. Determination of ampicillin in biological fluids by coupled-column liquid chromatography and post-column derivatization. *J.Chromatogr.*, **1991**, 567, 121-128

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. Filter using Molcut II (Millipore), inject a 50 μL aliquot of the ultrafiltrate. Urine. Dilute ten-fold with water, filter (Gelman acrylate copolymer 0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 4 \times 4 5 μm LiChrospher RP-18(e)

Column: 250 \times 4 5 μm LiChrospher RP-18(e)

Mobile phase: MeOH:20 mM tetrabutylammonium bromide + 5 mM Na_2HPO_4 + 5 mM NaH_2PO_4 1:1.75

Flow rate: 0.8

Injection volume: 20-50

Detector: UV 270 following post-column reaction. The column effluent mixed with 2 M NaOH and 0.05% sodium hypochlorite solution pumped at 0.1 mL/min in a 400 \times 0.5 mm hollow fiber membrane reactor at 40° and this mixture flowed through a 1400 \times 0.3 mm knitted open tubular reactor at 50° to the detector.

CHROMATOGRAM

Retention time: 30

Limit of detection: 20 ng

OTHER SUBSTANCES

Extracted: sulbactam

KEY WORDS

post-column reaction; serum

REFERENCE

Haginaka, J.; Nishimura, Y. Simultaneous determination of ampicillin and sulbactam by liquid chromatography: post-column reaction with sodium hydroxide and sodium hypochlorite using an active hollow-fiber membrane reactor. *J.Chromatogr.*, **1990**, 532, 87-94

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 200 μ L Serum + 100 μ L 10 M urea, ultrafilter with Amicon YMT membrane at 1500 g for 10 min. 200 μ L Ultrafiltrate + 200 μ L 0.1 M pH 9 borate buffer + 20 μ L 0.2 M acetic anhydride solution in MeCN. Stand at RT for 3 min, add 400 μ L reagent, heat at 60° in a water bath for 10 min, cool, inject a 40-80 μ L aliquot. Urine. Dilute urine 10 fold with water and filter through 0.45 μ m acrylate copolymer membrane. 200 μ L Filtrate + 200 μ L 0.1 M pH 9 borate buffer + 20 μ L 0.2 M acetic anhydride solution in MeCN. Stand at RT 3 min, add 400 μ L reagent, heat at 60° in a water bath for 10 min, cool, inject a 20-80 μ L aliquot. (Reagent was 13.81 g 1,2,4-triazole in 60 mL water + 10 mL HgCl₂ solution (0.27 g HgCl₂ in 100 mL water), adjust pH to 9.0 \pm 0.05 with 4 M NaOH, dilute to 100 mL.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Develosil ODS-5 (Nomura Chemicals)

Mobile phase: MeCN:20 mM NaH₂PO₄:20 mM sodium thiosulfate:MeCN 25:37.5:37.5

Flow rate: 0.8

Injection volume: 20-80

Detector: UV 328

CHROMATOGRAM

Retention time: 6

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Also analyzed: amoxicillin, cyclacillin (ciclacillin)

KEY WORDS

derivatization; serum

REFERENCE

Haginaka, J.; Wakai, J. High-performance liquid chromatographic assay of ampicillin, amoxicillin and ciclacillin in serum and urine using a pre-column reaction with 1,2,4-triazole and mercury(II) chloride. *Analyst*, **1985**, *110*, 1277-1281

SAMPLE

Matrix: bulk, formulations

Sample preparation: Prepare a solution of capsule contents or bulk drug in the mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4 5 μ m LiChrospher RP-18

Mobile phase: MeCN:1% acetic acid 39:61

Flow rate: 2

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: degradation products, dicloxacillin

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

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Bakerbond C18**Mobile phase:** MeCN:water:1 M KH₂PO₄:1 M acetic acid 80:909:10:1**Flow rate:** 0.8**Detector:** UV 254

CHROMATOGRAM**Retention time:** 3.29

KEY WORDS

injections; saline; water; stability-indicating

REFERENCEStiles, M.L.; Allen, L.V., Jr.; Prince, S.J. Stability of various antibiotics kept in an insulated pouch during administration via portable infusion pump. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 70–74

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:** MeCN:MeOH:10 mM pH 2.6-2.7 phosphate buffer 7:14:79 containing 5 mM tetrabutylammonium hydrogen sulfate**Flow rate:** 1**Injection volume:** 20**Detector:** UV 225

CHROMATOGRAM**Retention time:** 4.35**Internal standard:** cimetidine (3.27)

OTHER SUBSTANCES**Simultaneous:** aztreonam, sulbactam

KEY WORDS

stability-indicating; saline; injections

REFERENCEBelliveau, 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:** Dilute injection with mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** μBondapak C18**Mobile phase:** MeCN:buffer 17.5:82.5 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 5.0 with concentrated phosphoric acid.)

Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: sulbactam

KEY WORDS
injections

REFERENCE

Mushinsky, R.F.; Reynolds, M.L.; Nicholson, C.A.; Crider, L.L.; Forcier, G.A. Stability of sulbactam/ampicillin in diluents for parenteral administration. *Rev.Infect.Dis.*, **1986**, 8 Suppl 5, S523-S527

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 μ L aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 70 mm long Co:Pell ODS

Column: 300 \times 4.6 10 μ m 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: 4.0

Limit of detection: 699 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin, cloxacillin, 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: 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 μ m polyvinylidene difluoride). Inject 2 mL into an LC system (150 \times 4.6 5 μ m 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 ampicillin (16.5 min), add 100 μ L reagent, evaporate to 1 mL, inject a 200 μ L aliquot. (Reagent was 10 mM phosphoric acid, 10 mM KH_2PO_4 , and 10 mM sodium decanesulfonate.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-18

Mobile phase: MeCN:buffer 33:67 (Buffer was 67 mM phosphoric acid, 3.3 mM KH_2PO_4 , and 5 mM sodium dodecyl sulfate.)

Flow rate: 1

Injection volume: 200

Detector: UV 210

CHROMATOGRAM

Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES

Also analyzed: amoxicillin, ceftiofur, cephalirin, cloxacillin, penicillin G, 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 μ L portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μ m), 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 \times 4.6 10 μ m 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; Charm II assay

CHROMATOGRAM

Retention time: 7.19

OTHER SUBSTANCES

Extracted: ceftiofur, cephalirin, 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. *JAOAC Int.*, **1995**, *78*, 1165–1172

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 μ m nylon). Inject 50 μ L 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 \times 8 Radial-Pak 10 μ m μ Bondapak 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, thin layer cell, 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: 3.2

Limit of detection: 0.3 ppm

OTHER SUBSTANCES

Simultaneous: cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V

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: 500 μ L Milk + 500 μ L 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 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 220 \times 2.1 5 μ m Spheri-5 phenyl microbore (UV detection) or 220 \times 4.6 5 μ m Spheri-5 phenyl microbore (MS detection)

Mobile phase: MeCN:85% phosphoric acid:triethylamine:water 20:0.4:0.4:79.2 containing 5 mM dodecanesulfonate (UV) or isopropanol:acetic acid in 200 mM ammonium acetate:water 10:2:88 (MS)

Column temperature: 50

Flow rate: 0.2-0.45 (UV) or 0.8-1.2 (MS)

Injection volume: 10-100

Detector: UV 220; MS, Finnigan MAT 4800 quadrupole, thermospray, source 320°, vaporizer 120°, pulsed positive ion negative ion

CHROMATOGRAM

Retention time: 8.3 (UV), 14.5 (MS)

Limit of detection: 200 ng/mL (MS)

OTHER SUBSTANCES

Also analyzed: amoxicillin, cloxacillin

KEY WORDS

ultrafiltrate; LC-MS

REFERENCE

Voysksner, 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: Condition a Sep-Pak C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 2 mL 2% NaCl. Pass through 30 g filtered (glass-wool plug) milk at 2 mL/min, wash with 5 mL water, wash with 10 mL MeOH:water:20% NaCl 10:80:10 containing 20 mM 18-crown-6, elute with 10 mL 15% (v/v) MeOH, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: 50 \times 2.1 Permaphase ETH (Du Pont)

Column: 150 \times 4.3 LiChrosorb RP-18

Mobile phase: MeOH:water:0.2 M pH 4.0 phosphate buffer 25:65:10 containing 11 mM sodium 1-heptanesulfonate

Column temperature: 45

Flow rate: 1

Injection volume: 100

Detector: UV 210

CHROMATOGRAM

Retention time: 11

Limit of detection: 30 ng/g

OTHER SUBSTANCES

Extracted: penicillin G, penicillin V

KEY WORDS

cow; SPE

REFERENCE

Terada, H.; Sakabe, Y. Studies on residual antibacterials in foods. IV. Simultaneous determination of penicillin G, penicillin V and ampicillin in milk by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, *348*, 379–387

SAMPLE

Matrix: reaction mixtures

Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 4.6 5 μ m Microsorb C8

Column: 250 \times 4.6 5 μ m Microsorb C8

Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 23:77

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 6.8

Limit of detection: 1 $\mu\text{g/mL}$

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 \times 3.9 10 μm μ Bondapak 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: 7.5

OTHER SUBSTANCES

Simultaneous: amoxicillin, cefaclor, cephalixin, cephadrine

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: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: present but not specified

Column: 150 \times 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: 4.5

OTHER SUBSTANCES

Simultaneous: amoxicillin, cephapirin

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

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- Straub, R.; Linder, M.; Voyksner, R.D. Determination of β -lactam residues in milk using perfusive-particle liquid chromatography combined with ultrasonic nebulization electrospray mass spectrometry. *Anal. Chem.*, **1994**, *66*, 3651–3658 [milk; electrospray; LC-MS; microbore; LOD 10 ppb; extracted amoxicillin, ceftiofur, cephapirin, cloxacillin, penicillin G]
- Tyczkowska, K.L.; Voyksner, R.D.; Straub, R.F.; Aronson, A.L. Simultaneous multiresidue analysis of β -lactam antibiotics in bovine milk by liquid chromatography with ultraviolet detection and confirmation by electrospray mass spectrometry. *J.AOAC Int.*, **1994**, *77*, 1122–1131 [cow; milk; LC-MS; UV detection; LOD 10 ppb; column temp 40; extracted amoxicillin, ceftiofur, cephapirin, cloxacillin, penicillin G]
- Akhtar, M.J.; Khan, S.; Khan, M.A. Determination of ampicillin in human plasma by high-performance liquid chromatography using ultraviolet detection. *J.Pharm.Biomed.Anal.*, **1993**, *11*, 375–378
- Kaniou, I.P.; Zachariadis, G.A.; Stratis, J.A. Separation and determination of five penicillins by reversed phase HPLC. *J.Liq.Chromatogr.*, **1993**, *16*, 2891–2897 [simultaneous amoxicillin, cloxacillin, dicloxacillin, penicillin G]
- Straub, R.F.; Voyksner, R.D. Determination of penicillin G, ampicillin, amoxicillin, cloxacillin and cephapirin by high-performance liquid chromatography-electrospray mass spectrometry. *J. Chromatogr.*, **1993**, *647*, 167–181 [milk; extracted amoxicillin, cephapirin, cloxacillin, penicillin G; LC-MS; electrospray; UV detection; LOD 100 ppb]
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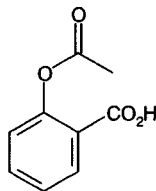
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Aspirin

Molecular formula: C₉H₈O₄

Molecular weight: 180.2

CAS Registry No.: 50-78-2



SAMPLE

Matrix: blood

Sample preparation: Plasma. 200 μ L Plasma + 200 μ L 5 μ g/mL IS in 200 mM HCL:200 mM orthophosphoric acid 50:50, vortex for 1-2 s, add 400 μ L MeCN, vortex, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 100-120 mg NaCl, vortex briefly, let stand at 4° for 10 min, vortex, centrifuge at 10500 g for 1 min, inject a 10 μ L aliquot of the upper organic layer. Whole blood. 200 μ L Lysed whole blood + 400 μ L 5 μ g/mL IS in 200 mM HCL:200 mM orthophosphoric acid 50:50, vortex for 1-2 s, add 600 μ L MeCN, vortex, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 200 mg NaCl, vortex briefly, let stand at 4° for 10 min, vortex, centrifuge at 10500 g for 1 min, inject a 10 μ L aliquot of the upper organic layer.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Novapak C18

Mobile phase: MeCN:water:85% orthophosphoric acid 18:74:0.09 (Before use prime column by recycling 200 mL mobile phase + 400 μ L di-n-butylamine overnight at 0.3 mL/min.)

Column temperature: 30

Flow rate: 1

Injection volume: 10

Detector: UV 237

CHROMATOGRAM

Retention time: 4.2

Internal standard: 2-methylbenzoic acid (8.9)

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, gentisic acid, salicylic acid, salicyluric acid

KEY WORDS

plasma; whole blood; pharmacokinetics

REFERENCE

Kees, F.; Jehnich, D.; Grobecker, H. Simultaneous determination of acetylsalicylic acid and salicylic acid in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1996**, 677, 172-177

SAMPLE

Matrix: blood

Sample preparation: Add o-anisic acid to 1 mL plasma, acidify with HCl, extract with diethyl ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-8

Mobile phase: MeCN:20 mM phosphoric acid 15:85

Flow rate: 1.6

Detector: UV 237

CHROMATOGRAM**Internal standard:** o-anisic acid**Limit of quantitation:** 20 ng/mL

OTHER SUBSTANCES**Extracted:** salicylic acid

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Benedek, I.H.; Joshi, A.S.; Pieniaszek, H.J.; King, S.-Y.P.; Kornhauser, D.M. Variability in the pharmacokinetics and pharmacodynamics of low dose aspirin in healthy male volunteers. *J.Clin. Pharmacol.*, **1995**, *35*, 1181-1186

SAMPLE**Matrix:** blood**Sample preparation:** Filter (0.22 μ m), inject a 2 μ L aliquot of the filtrate.

HPLC VARIABLES**Column:** 250 \times 4.5 μ m LiChrospher 100 Diol**Mobile phase:** MeCN:50 mM pH 3.0 phosphate buffer 1.5:98.5**Flow rate:** 0.5**Injection volume:** 2**Detector:** UV 254

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES**Extracted:** salicylic acid

KEY WORDS

direct injection; serum

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:** 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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 μ m 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: 3.40
Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amitriptyline, amodiaquine, amoxapine, astemizole, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpi-pramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazeponide, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, nadolol, nalbuphine, nalorphine, naloxone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, reserpine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulphide, suriclone, temazepam, tenoxicam, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: albuterol, amisulpride, atenolol, chlormezanone, codeine, lisinopril, metformin, naltrexone, phenobarbital, phenol, ranitidine, ritodrine, sultopride, terbutaline, tiapride, toloxatone

KEY WORDS

whole blood; plasma

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 + 0.2 mL 1 M HCl + 10 mL diethyl ether, gently mix for 10 min, centrifuge at 1500 rpm for 4 min. Remove the organic phase, evaporate it to dryness at 0° under a stream of nitrogen, add 200 µL mobile phase, vortex 90 s, inject a 5-100 µL aliquot.

HPLC VARIABLES**Guard column:** 23 × 3.9 μBondapak C18/Porasil B**Column:** 300 × 3.9 10 μm μBondapak C18**Mobile phase:** MeOH:water:1-butanol:orthophosphoric acid 270:720:10:0.13**Column temperature:** 47**Flow rate:** 1.8**Injection volume:** 5-100**Detector:** UV 234

CHROMATOGRAM**Retention time:** 5.6**Internal standard:** m-anisic acid (9.6)**Limit of quantitation:** 10-15 ng/mL

OTHER SUBSTANCES**Simultaneous:** salicylic acid**Noninterfering:** acetaminophen, albuterol, aminophylline, amitriptyline, atenolol, beclomethasone, bromazepam, caffeine, carbamazepine, chloral hydrate, chlordiazepoxide, cimetidine, clonazepam, codeine, desipramine, dexamethasone, dextropropoxyphene, diazepam, dicyclomine, digoxin, disopyramide, doxycycline, ergotamine, ethosuximide, furosemide, gentisic acid, haloperidol, hydrocortisone, imipramine, indomethacin, levodopa, lignocaine, lithium carbonate, meperidine, methdilazine, methylphenobarbitone, methylprednisolone, methysergide, metoclopramide, metoprolol, mexiletine, midazolam, naphthoxyacetic acid, nitrazepam, nitroglycerin, nortriptyline, oxazepam, oxpranolol, pentobarbitone, pethidine, phenytoin, prednisolone, prednisone, primidone, procainamide, prochlorperazine, propranolol, quinidine, salicylic acid, spironolactone, sulfamethoxazole, theophylline, trimethoprim, valproic acid, verapamil, warfarin**Interfering:** methylothiazide

KEY WORDS

plasma; pharmacokinetics

REFERENCEBrandon, R.A.; Eadie, M.J.; Smith, M.T. A sensitive liquid chromatographic assay for plasma aspirin and salicylate concentrations after low doses of aspirin. *Ther. Drug Monit.*, **1985**, *7*, 216-221

SAMPLE**Matrix:** blood**Sample preparation:** 50 μL Serum + 50 μL 15 μg/mL β-hydroxyethyltheophylline in MeCN, mix for 30 s, centrifuge at 13000 g for 5 min. Inject the supernatant (about 20 μL).

HPLC VARIABLES**Column:** μBondapak C18**Mobile phase:** MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH₂PO₄ adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean column with water for 20 min and MeOH for 30 min.)**Flow rate:** 2**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 12**Internal standard:** β-hydroxyethyltheophylline (5.8)**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Extracted:** salicylic acid**Simultaneous:** acetaminophen, acetylprocainamide, caffeine, dyphylline, procainamide, theophylline**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:** Mix plasma with an equal volume of MeCN, vortex for 30 s, centrifuge at 900 g for 5 min, inject a 100 μ L aliquot of the supernatant.**HPLC VARIABLES****Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeOH:acetic acid:water 22:5:73**Flow rate:** 2.6**Injection volume:** 100**Detector:** UV 280**CHROMATOGRAM****Retention time:** 4.75**Limit of quantitation:** 2 μ g/mL**OTHER SUBSTANCES****Extracted:** salicylic acid**Noninterfering:** acetaminophen, albuterol, aminophylline, amitriptyline, amoxicillin, ampicillin, amobarbital, beclomethasone, carbamazepine, carbenicillin, chlordiazepoxide, cimetidine, clonazepam, cyproheptadine, debrisoquine, dextropropoxyphene, diazepam, digoxin, dihydroxyanthraquinone, ergotamine, ethosuximide, fluphenazine, furosemide, gentamicin, gentisic acid, guaifenesin, haloperidol, heparin, hydrocortisone, indomethacin, methdilazine, methyclothiazide, methylphenobarbitone, methysergide, metoclopramide, naproxen, nitrazepam, nystatin, penicillin, pentobarbitone, phenytoin, phenytoin, pizotifen, prazosin, prednisone, prochlorperazine, propranolol, spironolactone, sulfamethoxazole, theophylline, trifluoperazine, trimethoprim, valproic acid**KEY WORDS**

plasma

REFERENCE

Cham, B.E.; Ross-Lee, L.; Bochner, F.; Imhoff, D.M. Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay. *Ther.Drug Monit.*, **1980**, *2*, 365-372

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 500 μ L Plasma + 900 μ L 270 mM HCl + 100 μ L 100 μ g/mL α -phenylcinnamic acid in MeOH + 10 mL dichloromethane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to

dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot. Urine. 2 mL Urine + 900 μL 270 mM HCl + 100 μL 100 $\mu\text{g}/\text{mL}$ α -phenylcinnamic acid in MeOH + 10 mL hexane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 300 \times 4 μm Bondapak C18
Mobile phase: MeOH:1% acetic acid 60:40
Flow rate: 2
Injection volume: 25
Detector: UV 280

CHROMATOGRAM

Retention time: 2.5
Internal standard: α -phenylcinnamic acid (8.0)
Limit of detection: 2 $\mu\text{g}/\text{mL}$

OTHER SUBSTANCES

Extracted: salicylic acid (UV 300), salsalate (UV 300)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Harrison, L.I.; Funk, M.L.; Ober, R.E. High-pressure liquid chromatographic determination of salicylic acid, aspirin, and salicylic acid in human plasma and urine. *J.Pharm.Sci.*, **1980**, *69*, 1268-1271

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Prepare a 20 mg/mL solution of bulk aspirin in dichloromethane, inject a 10 μL aliquot as soon as dissolution is complete. Tablets. Prepare a 20 mg/mL solution of ground aspirin tablets in dichloromethane, filter (0.45 μm) immediately, immediately inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 \times 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:acetic acid 80:19:3 (Before first use pump 10 column volumes of dichloromethane:acetic acid:2,3-dimethoxypropane 96:2:2 through column at 3 mL/min.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Simultaneous: impurities, salicylic acid, salsalate

KEY WORDS

normal phase; tablets

REFERENCE

Pfeiffer, C.D.; Pankey, J.W. Determination of related compounds in aspirin by liquid chromatography. *J.Pharm.Sci.*, **1982**, *71*, 511-514

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

REFERENCEDi 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:** formulations**Sample preparation:** Condition a 500 mg Extract Clean silica SPE cartridge (Alltech stock no. 209250) with 2 mL hexane. Allow a solution of aspirin in 10 mM sorbitan trioleate in CFC-11 to evaporate, dissolve the residue in 5 mL hexane. Add 1 mL to the SPE cartridge, elute with two 2 mL portions of mobile phase, make up eluate to 5 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES**Guard column:** 20 × 2 30-40 μm Perisorb RP-8 Pellicular (Upchurch)**Column:** 250 × 4.6 5 μm Econosphere C8**Mobile phase:** MeOH:THF:1 M phosphoric acid:water 44:5:5:46**Flow rate:** 1**Injection volume:** 20**Detector:** UV 275

CHROMATOGRAM**Retention time:** 5.6

OTHER SUBSTANCES**Extracted:** degradation products, salicylic acid

KEY WORDS

aerosols; SPE

REFERENCE

Blondino, F.E.; Byron, P.R. The quantitative determination of aspirin and its degradation products in a model solution aerosol. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 111–119

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, 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 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 10 μ m 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: 2.5

Internal standard: pyrimethamine (3.5)

Limit of quantitation: 6 μ g/mL

OTHER SUBSTANCES

Simultaneous: dipyridamole

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: formulations

Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μ L aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μ m), discard first 5 mL of filtrate, inject a 10 μ L aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μ m), discard first 5 mL of filtrate, inject a 10 μ L aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax ODS

Mobile phase: MeOH:water 75:25

Flow rate: 1.5

Injection volume: 10

Detector: UV 240

CHROMATOGRAM**Retention time:** 2.5**Limit of detection:** 5 µg/mL

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, benzyl benzoate, boldenone, calusterone, cortisone, dehydroepiandrosterone (UV 210), ethisterone, fluoxymesterone, mesterolone (UV 210), methandriol (UV 210), methandrostenolone, methenolone acetate, methyltestosterone, mibolerone, nandrolone, nandrolone acetate, nandrolone propionate, norethandrolone, norethindrone, norethindrone acetate, norgestrel, oxandrolone (UV 210), oxymetholone, stanozolol, testosterone, testosterone acetate, testosterone propionate, trenbolone acetate

Interfering: caffeine, formebolone, testolactone

KEY WORDSoils; 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: Place 5 tablets in MeCN:MeOH:85% phosphoric acid 92:8:0.5, sonicate 15 min, shake 15 min, dilute to 250 mL. Centrifuge an aliquot in 50 mL tube at 2000 rpm for 15 min and filter supernatant (0.45 µm).

HPLC VARIABLES**Column:** 150 × 3.9 Resolve (Waters)**Mobile phase:** MeCN:water:85% phosphoric acid 24:76:0.5**Column temperature:** 35**Flow rate:** 2**Injection volume:** 10**Detector:** UV 295

CHROMATOGRAM**Retention time:** 2.2

OTHER SUBSTANCES

Simultaneous: acetaminophen, caffeine, salicylic acid

KEY WORDSfilm coated tablets; tablets

REFERENCE

Fogel, J.; Epstein, P.; Chen, P. Simultaneous high-performance liquid chromatography assay of acetylsalicylic acid and salicylic acid in film-coated aspirin tablets. *J.Chromatogr.*, **1984**, *317*, 507–511

SAMPLE**Matrix:** formulations

Sample preparation: Grind tablets to a fine powder and add about 250 mg aspirin to 100 mL chloroform saturated with citric acid containing 500 µL formic acid, add 500 mg solid citric acid, sonicate for 2 min, centrifuge or filter, inject an aliquot. (If buffers or antacid are present, add ground tablets equivalent to about 500 mg aspirin to 3 g acid-washed siliceous earth, mix, add 2 mL 6 M HCl, mix, add to a 200 × 25 column, dry wash

container with siliceous earth, add to column, elute column with chloroform saturated with citric acid at 10 mL/min. Collect 150 mL eluent, add 1 mL formic acid, make up to 200 mL with chloroform saturated with citric acid, add 500 mg citric acid, shake, inject an aliquot.)

HPLC VARIABLES

Column: 250 × 4.6 5 μ m Zorbax-Sil

Mobile phase: Chloroform:dichloromethane:acetonitrile:formic acid 700:300:30:4 (At the end of the day wash the column with 200 mL MeOH.)

Flow rate: 2

Injection volume: 20

Detector: UV 300

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: acetylsalicylic acid anhydride, acetylsalicylsalicylic acid, salicylic acid, excipients

KEY WORDS

normal phase; tablets; SPE

REFERENCE

Galante, R.N.; Visalli, A.J.; Grim, W.M. Stabilized normal-phase high-performance liquid chromatographic analysis of aspirin and salicylic acid in solid pharmaceutical dosage forms. *J.Pharm.Sci.*, 1984, 73, 195-197

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: 24

Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES

Simultaneous: acetaminophen, O-acetyl-p-aminophenol, 2-O-acetylascorbic acid, 3-O-acetylascorbic acid, p-aminophenol, diacetyl-p-aminophenol (UV 280), saccharin, salicylic acid (UV 280), vitamin C

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: solutions

HPLC VARIABLES

Column: 250 × 4.1 6 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 32:68

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: diazepam, diphenhydramine, o-hydroxyhippuric acid, MPPH, niacin, toluene

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: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)

Mobile phase: MeCN:water 20:80

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: acetaminophen, phenacetin, salicylamide

REFERENCE

Jedrejewski, P.T.; Taylor, L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography. *J.Chromatogr.Sci.*, **1995**, *33*, 438–445

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 KH₂PO₄ and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 231

CHROMATOGRAM**Retention time:** 3.4**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: carbamazepine, chlordiazepoxide, 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

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, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, 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, fenopropfen, 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, isocarboxystyryl, 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, methylidopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, na-

proxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylicrin, 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, pyriline, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, zaccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopolamine, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, 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 50 µg/mL solution in the mobile phase, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 µm Lichrosorb RP 18

Mobile phase: MeOH:water 45:55 containing 1% acetic acid

Flow rate: 1

Injection volume: 10

Detector: UV 230

CHROMATOGRAM

Retention time: 5.09

OTHER SUBSTANCES

Simultaneous: acetaminophen, phenacetin, salicylamide, salicylic acid

REFERENCE

Nivaud-Guernet, E.; Guernet, M.; Ivanovic, D.; Medenica, M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography. *J. Liq. Chromatogr.*, **1994**, *17*, 2343-2357

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:** 3

OTHER SUBSTANCES**Simultaneous:** carprofen, diflunisal, fenbufen, ibuprofen, indomethacin, naproxen, tolmetin

REFERENCESlingsby, 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 compounds in MeOH, inject a 1 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 1.3 μ m 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:** 3.52

OTHER SUBSTANCES**Also analyzed:** acetaminophen, bucetin (3-hydroxy-p-butyrophenetidine), caffeine, dipyrrone (sulpyrin), ethenzamide (o-ethoxybenzamide), mefenamic acid, phenacetin, salicylamide, salicylic acid, theobromine, theophylline

KEY WORDS

semi-micro; porous polymer

REFERENCEMatsushima, Y.; Nagata, Y.; Niyomura, M.; Takakusagi, K.; Takai, N. Analysis of antipyretics by semi-micro liquid chromatography. *J.Chromatogr.*, **1985**, *332*, 269–273

SAMPLE**Matrix:** urine**Sample preparation:** Acidify 5 mL urine to pH 1 with 40% phosphoric acid, shake with two 5 mL aliquots of diethyl ether, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL MeOH, inject a 25 μ L aliquot.

HPLC VARIABLES**Column:** μ Bondapak C18 radial compression**Mobile phase:** MeCN:0.085% phosphoric acid 20:80**Flow rate:** 1.5**Injection volume:** 25**Detector:** UV 237

CHROMATOGRAM**Limit of detection:** 500 ng/mL

KEY WORDS

horse

REFERENCE

Beaumier, P.M.; Fenwick, J.D.; Stevenson, A.J.; Weber, M.P.; Young, L.M. Presence of salicylic acid in standardbred horse urine and plasma after various feed and drug administrations. *Equine.Vet.J.*, **1987**, *19*, 207–213

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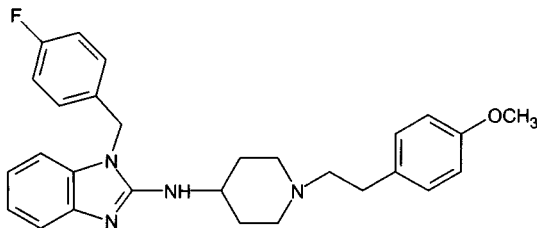
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- Wongyai, S. Synthesis and characterization of phenylpropanolamine bonded silica for multimode liquid chromatography of small molecules. *Chromatographia*, **1994**, *38*, 485–490 [simultaneous benzoic acid, salicylic acid]
- Hays, P.A.; Lurie, I.S. Quantitative analysis of adulterants in illicit heroin samples via reversed phase HPLC. *J.Liq.Chromatogr.*, **1991**, *14*, 3513–3517 [simultaneous acetaminophen, acetylcodeine, acetylmorphine, benzocaine, caffeine, chloroquine, diamorphine, diazepam, diphenhydramine, dipyrone, lidocaine, methaqualone, monoacetylmorphine, morphine, nicotinamide, noscapine, papaverine, phenacetin, phenobarbital, phenolphthalein, N-phenyl-2-naphthylamine, salicylic acid, strychnine]
- Shen, J.; Wanwimolruk, S.; Clark, C.R.; Roberts, M.S. A sensitive assay for aspirin and its metabolites using reversed-phase ion-pair high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1990**, *13*, 751–761 [simultaneous metabolites gentisic acid, salicylic acid; LOD 50 ng/mL]
- Lam, S.; Malikin, G. An improved micro-scale protein precipitation procedure for HPLC assay of therapeutic drugs in serum. *J.Liq.Chromatogr.*, **1989**, *12*, 1851–1872 [serum; also acetaminophen, amiodarone, caffeine, chloramphenicol, flecainide, pentobarbital, procainamide, pyrimethamine, quinidine, theophylline, tocainide, trazodone; fluorescence detection; UV detection]
- Lurie, I.S.; McGuinness, K. The quantitation of heroin and selected basic impurities via reversed phase HPLC. II. The analysis of adulterated samples. *J.Liq.Chromatogr.*, **1987**, *10*, 2189–2204 [UV detection; electrochemical detection; simultaneous acetaminophen, acetylcodeine, acetylmorphine, acetylprocaine, aminopyrene, amitriptyline, antipyrine, barbital, benzotropine, caffeine, cocaine, codeine, diamorphine, diazepam, diphenhydramine, dipyrone, ephedrine, ethylmorphine, lidocaine, meconin, methamphetamine, methapyrilene, methaqualone, monoacetylmorphine, morphine, nalorphine (IS), niacinamide, noscapine, papaverine, phenacetin, phenmetrazine, phenobarbital, phenolphthalein, procaine, propanophenone, propoxyphene, pyrilamine, quinidine, quinine, salicylamide, salicylic acid, secobarbital, strychnine, tartaric acid, tetracaine, thebaine, tripelellamine, tropacocaine, vitamin B₆, vitamin B₁₂]
- Lau, A.H.; Chang, C.W.; Schlesinger, P.K. Evaluation of a potential drug interaction between sucralfate and aspirin. *Clin.Pharmacol.Ther.*, **1986**, *39*, 151–155 [plasma; pharmacokinetics; extracted metabolites, salicylic acid, salicylic acid; α -phenylcinnamic acid (IS); gradient; column temp 30]
- Mamolo, M.G.; Vio, L.; Maurich, V. High-pressure liquid chromatographic analysis of paracetamol, caffeine and acetylsalicylic acid in tablets. Salicylic acid quantitation. *Farmaco.[Prat.]*, **1985**, *40*, 111–123 [tablets; simultaneous acetaminophen, caffeine, phenazone, salicylic acid]
- Pedersen, A.K.; FitzGerald, G.A. Preparation and analysis of deuterium-labeled aspirin: application to pharmacokinetic studies. *J.Pharm.Sci.*, **1985**, *74*, 188–192 [stability; simultaneous salicylic acid]
- Bevitt, R.N.; Mather, J.R.; Sharman, D.C. Minimization of salicylic acid formation during preparation of aspirin products for analysis by high-performance liquid chromatography. *Analyst*, **1984**, *109*, 1327–1329
- Sreenivasan, K.; Nair, P.D.; Rathinam, K. A GPC method for analysis of low molecular weight drugs. *J.Liq.Chromatogr.*, **1984**, *7*, 2297–2305 [GPC; SEC; simultaneous salicylic acid]
- Das Gupta, V. Simultaneous quantitation of acetaminophen, aspirin, caffeine, codeine phosphate, phenacetin, and salicylamide by high-pressure liquid chromatography. *J.Pharm.Sci.*, **1980**, *69*, 110–113
- Kirchhoefer, R.D. Simultaneous determination of aspirin and salicylic acid in bulk aspirin and in plain, buffered, and enteric-coated tablets by high-pressure liquid chromatography with UV and fluorescence detectors. *J.Pharm.Sci.*, **1980**, *69*, 1188–1191

Astemizole

Molecular formula: C₂₈H₃₁FN₄O

Molecular weight: 458.6

CAS Registry No.: 68844-77-9



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)) KH₂PO₄ 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: 5.73

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpromazine, chlorpropamide, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glipizide, glutethimide, haloperidol, histapyrridine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loratadine, lorazepam, loxapine, maprotiline, medazepam, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephesisin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, pen-

fluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vincristine, vindsesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: bisoprolol, cetirizine, chlorpheniramine, cibenzoline, diltiazem, glibornuride, loprazolam, medifoxamine, moperone, nicardipine, vinblastine

KEY WORDS

whole blood; plasma

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: Plasma. 2 mL Plasma + 100 μ L 1 μ g/mL IS in MeOH + 2 mL 50 mM borax solution + 4 mL heptane:isoamyl alcohol 95:5, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 5 min. Remove upper organic layer and repeat extraction of aqueous layer. Combine extracts, add 3 mL 50 mM sulfuric acid, extract. Remove acidic aqueous phase and make it alkaline with concentrated ammonia, extract twice with 2 mL heptane:isoamyl alcohol 95:5, combine organic layers and evaporate them to dryness under a stream of nitrogen at 55°, take up in 50 μ L MeOH, inject the whole sample. Tissue. Grind tissue in a Waring blender, homogenize 1:4 in water. 2 mL homogenate + 100 μ L 1 μ g/mL IS in MeOH + 2 mL 50 mM borax solution + 4 mL heptane:isoamyl alcohol 95:5, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 5 min. Remove upper organic layer and repeat extraction of aqueous layer. Combine extracts, add 3 mL 50 mM sulfuric acid, extract. Remove acidic aqueous phase and make it alkaline with concentrated ammonia, extract twice with 2 mL heptane:isoamyl alcohol 95:5, combine organic layers and evaporate them to dryness under a stream of nitrogen at 55°, take up in 50 μ L MeOH, inject the whole sample.

HPLC VARIABLES

Column: 150 \times 2.1 5 μ m Alltech RSiL C18HL

Mobile phase: MeCN:water 50:50 + 0.05% diethylamine

Flow rate: 0.6

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 5.8

Internal standard: 1-[(4-fluorophenyl)methyl]-N-{1-[2-(4-ethoxyphenyl)ethyl]-4-piperidinyl}-1H-benzimidazol-2-amine (R 44 180) (8.3)

Limit of detection: 1 ng/mL (plasma); 5 ng/g (tissue)

OTHER SUBSTANCES

Extracted: metabolites, desmethylastemizole

KEY WORDS

plasma; human; dog

REFERENCE

Woestenborghs, R.; Embrechts, L.; Heykants, J. Simultaneous determination of astemizole and its demethylated metabolite in animal plasma and tissues by high-performance liquid chromatography. *J.Chromatogr.*, **1983**, *278*, 359–366

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 150 × 4.6 5 μm MicroPack MCH-5

Mobile phase: MeOH:30 mM pH 3.0 phosphate buffer 85:15

Flow rate: 1.5

Injection volume: 20

Detector: UV 276

REFERENCE

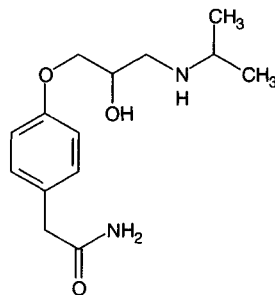
Fernández Otero, G.C.; Lucangioli, S.E.; Carducci, C.N. Adsorption of drugs in high-performance liquid chromatography injector loops. *J.Chromatogr.A*, **1993**, *654*, 87–91

Atenolol

Molecular formula: C₁₄H₂₂N₂O₃

Molecular weight: 266.3

CAS Registry No.: 29122-68-7



SAMPLE

Matrix: blood

HPLC VARIABLES

Column: 83 × 4.6 3 μm Pecosphere C18

Mobile phase: MeCN:MeOH:10 mM pH 4.0 sodium acetate containing 10 mM octanesulfonic acid 17:2:80.1

Detector: F ex 228 em 310

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Hartmann, D.; Stief, G.; Lingenfelder, M.; Güzelhan, C.; Horsch, A.K. Study on the possible interaction between tenoxicam and atenolol in hypertensive patients. *Arzneimittelforschung*, **1995**, *45*, 494–498

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL cyano SPE cartridge (J.T.Baker) with 2 mL water. 400 μL Serum + 20 μL practolol solution, vortex, add to the SPE cartridge, elute with 1 mL MeOH:triethylamine 99:1. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 80 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-8-OB

Mobile phase: MeCN:20 mM ammonium dihydrogen phosphate 6:94 containing 2% triethylamine, pH adjusted to 5.0

Flow rate: 1

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Retention time: 10

Internal standard: practolol (14)

Limit of detection: 50 ng/mL

KEY WORDS

serum; SPE; pharmacokinetics

REFERENCE

Phelps, S.J.; Alpert, B.S.; Ward, J.L.; Pieper, J.A.; Lima, J.J. Absorption pharmacokinetics of atenolol in patients with the Marfan syndrome. *J.Clin.Pharmacol.*, **1995**, *35*, 268–274

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 50 μ L 2 M KOH + 100 μ L pH 12.2 glycine buffer + 5 mg NaCl, vortex, add 8 mL dichloromethane:1-butanol 95:5, extract. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 1 mg/mL 4-dimethylaminopyridine in dioxane, add 500 μ L 20% phosphate in toluene, vortex, heat at 70° for 1 h, heat at 40° overnight. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4 LiChrosorb Si 100 modified with (R, R)-DACH-DNB (see J. Chromatogr. 1991, 539, 25)**Mobile phase:** Dichloromethane:MeOH 98:2**Flow rate:** 1**Injection volume:** 20**Detector:** F ex 230 em 300

CHROMATOGRAM**Internal standard:** (R, S)-n-pentyl propranolol hydrochloride**Limit of detection:** 0.5-0.6 ng/mL

KEY WORDS

plasma; chiral; derivatization

REFERENCEStoschitzky, K.; Kahr, S.; Donnerer, J.; Schumacher, M.; Luha, O.; Maier, R.; Klein, W.; Lindner, W. Stereoselective increase of plasma concentrations of the enantiomers of propranolol and atenolol during exercise. *Clin.Pharmacol.Ther.*, **1995**, 57, 543-551

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 μ m NovaPack C18**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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:** 3.33**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES**Extracted:** acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisul-

pride, amitriptyline, amodiaquine, amoxapine, astemizole, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzone, cicletamine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mepiridine, mephesisin, mephentermine, mepivacaine, metapramine, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, reserpine, secobarbital, sotalol, strychnine, sulindac, sultopride, suriclone, temazepam, tenoxicam, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: albuterol, aspirin, chlormezanone, flumazenil, metformin, morphine, phenobarbital, phenol, ranitidine, ritodrine, sulfinpyrazole, sulpride, terbutaline, tiapride, toloxatone

KEY WORDS

whole blood; plasma

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: Inject 150 μ L plasma onto column A with mobile phase A and elute to waste, after 11 min switch 10 mL of eluate containing atenolol onto column B. After 5 min elute column A to waste again and elute column B with mobile phase C onto column C, after 1.25 min, remove column B from the circuit, elute column C with mobile phase C, monitor the effluent from column C. When not in use column B is washed with mobile phase B.

HPLC VARIABLES

Column: A 100 \times 7.6 Asahipak GS220M size exclusion (Asahi Chemical); B 50 \times 4.6 Chemcosorb 7-ODS-H (Chemco Scientific); C 150 \times 6 Shinwa β -perphenylcarbamate bonded silica (Shinwa Chemical)

Mobile phase: A pH 7.5 phosphate buffer (I = 0.01); B water; C EtOH:20 mM pH 4.6 NaH₂PO₄ 10:90

Flow rate: A 2; B 1; C 1
Injection volume: 150
Detector: F ex 270 em 305

CHROMATOGRAM

Retention time: 19.8 ((S)-atenolol), 24.3 ((R)-atenolol)
Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; chiral; direct injection; column-switching; heart-cut

REFERENCE

He, J.; Shibukawa, A.; Nakagawa, T.; Wada, H.; Fujima, H.; Imai, E.; Go-oh, Y. Direct injection analysis of atenolol enantiomers in plasma using an achiral/chiral coupled column HPLC system. *Chem.Pharm.Bull.*, **1993**, *41*, 544–548

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Si SPE cartridge by washing twice with 1 mL MeOH, twice with 1 mL water, and once with 1 mL 100 mM pH 9.2 K_2HPO_4 . Add 1 mL plasma + 100 μ L water, wash twice with 1 mL water, centrifuge at 1000 g for 5 min, elute with 1 mL MeOH. Evaporate MeOH to dryness at 40° under a stream of air and dissolve residue in 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Spherisorb S5 SCX

Mobile phase: MeOH:MeCN:water 40:40:20 containing 0.2% perchloric acid (apparent pH 1.7)

Flow rate: 1.5

Injection volume: 100

Detector: F ex 200 no emission filter

CHROMATOGRAM

Retention time: 13

Internal standard: atenolol

OTHER SUBSTANCES

Extracted: albuterol, terbutaline

Noninterfering: aminophylline, beclomethasone, cloprednol, dexamethasone, fenoterol, ipratropium bromide, methylprednisolone, orciprenaline, prednisolone, reproterol, rimiterol, salmeterol, sodium cromoglycate, theophylline

KEY WORDS

plasma; SPE; atenolol is IS

REFERENCE

McCarthy, P.T.; Atwal, S.; Sykes, A.P.; Ayres, J.G. Measurement of terbutaline and salbutamol in plasma by high performance liquid chromatography with fluorescence detection. *Biomed.Chromatogr.*, **1993**, *7*, 25–28

SAMPLE

Matrix: blood

Sample preparation: 1 mL Whole blood + 1 mL 2.5 μ g/mL methoxamine in 100 mM NaOH, vortex, add 5 mL ethyl acetate, shake at 230 oscillations/min on a reciprocating shaker for 15 min, centrifuge at 2000 g for 15 min, repeat the extraction with 3 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of

nitrogen at 37°, reconstitute the residue in 50 µL 100 mM NaOH, vortex briefly, add 200 µL 200 mM (-)-menthyl chloroformate in MeCN, vortex for 30 s, let stand at room temperature for 10 min, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Hypersil ODS
Mobile phase: MeCN:MeOH:water 43:25:32
Flow rate: 1.2
Injection volume: 50
Detector: F ex 230 em 305

CHROMATOGRAM

Retention time: 9.8 (S-(-)), 11.0 (R-(+))
Internal standard: methoxamine (14.0, 14.8 (enantiomers))
Limit of quantitation: 12.5 ng/mL

KEY WORDS

derivatization; chiral; whole blood; pharmacokinetics

REFERENCE

Miller, R.B.; Guertin, Y. High-performance liquid chromatographic assay for the derivatized enantiomers of atenolol in whole blood. *J.Liq.Chromatogr.*, **1992**, *15*, 1289–1302

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 500 µL 10 M NaOH + 300 mg NaCl + 5 mL diethyl ether, shake for 10 min, centrifuge at 1500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 200 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm STR ODS-M (Shimadzu)
Mobile phase: MeCN:50 mM ammonium acetate adjusted to pH 4.5 with acetic acid 15:85
Column temperature: 35
Flow rate: 0.8
Injection volume: 50
Detector: F ex 230 em 300

CHROMATOGRAM

Retention time: 4
Internal standard: atenolol

OTHER SUBSTANCES

Extracted: nadolol

KEY WORDS

atenolol is IS; serum

REFERENCE

Noguchi, H.; Yoshida, K.; Murano, M.; Naruto, S. Determination of nadolol in serum by high-performance liquid chromatography with fluorimetric detection. *J.Chromatogr.*, **1992**, *573*, 336–338

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 5 μ L 100 μ g/mL racemic practolol in MeOH + 50 μ L 0.5 M pH 12 glycine buffer + 50 μ L 2 M NaOH + 1 mL saturated NaCl + 4 mL dichloromethane containing 3% (v/v) heptafluoro-1-butanol, extract for 10 min, centrifuge at 3015 g at 4° for 5 min, remove organic phase and evaporate to dryness at room temperature under a stream of nitrogen. Dissolve the residue in 40 μ L 1 M pH 8.5 borate buffer and 50 μ L 1 mM (+)-1-(9-fluorenyl)ethyl chloroformate (Flec) in acetone, let stand 30 min at room temperature, add 100 μ L 30 mM hydroxyproline, after 2 min vortex mix with 300 μ L n-pentane for 15 s, centrifuge at 3015 g at 4° for 10 min, discard n-pentane. Shake aqueous layer with 3 mL dichloromethane for 10 min, centrifuge at 3015 g at 4° for 10 min, remove organic phase and evaporate to dryness at room temperature under a stream of nitrogen. Dissolve the residue in 50 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Microspher C18 (Chrompack)

Mobile phase: MeCN:10 mM pH 7 sodium acetate buffer 50:50

Flow rate: 0.8

Injection volume: 10

Detector: F ex 227 em 310

CHROMATOGRAM

Retention time: 5.95 (S-(-)), 6.55 (R-(+))

Internal standard: practolol (8, 9)

Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; chiral; rat; derivatization

REFERENCE

Rosseel, M.T.; Vermeulen, A.M.; Belpaire, F.M. Reversed-phase high-performance liquid chromatographic analysis of atenolol enantiomers in plasma after chiral derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate. *J.Chromatogr.*, **1991**, 568, 239–245

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum or plasma + 50 μ L water + 100 μ L buffer + 5 mL dichloromethane:isopropanol, rotate at 40 rpm for 5 min, centrifuge at 800 g for 3 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μ L 50 mM sulfuric acid, vortex for 15 s, inject a 30-50 μ L aliquot. (Buffer was prepared by adjusting the pH of a saturated solution of disodium tetraborate to 9 with 6 M HCl.)

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m ODS Hypersil

Mobile phase: MeCN:10 mM pH 3.2 phosphate buffer 20:80 containing 3 mM sodium 1-octanesulfonate

Flow rate: 1

Injection volume: 30-50

Detector: UV 226

CHROMATOGRAM

Retention time: 4.0

Internal standard: atenolol

OTHER SUBSTANCES

Extracted: sotalol

KEY WORDS

serum; plasma; atenolol is IS

REFERENCE

Urech, R.; Chan, L.; Duffy, P. High-performance liquid chromatographic assay of sotalol: improved procedure and investigation of peak broadening. *J.Chromatogr.*, **1990**, *534*, 271–278

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond-Elut CN SPE cartridge with 2 mL MeOH then 2 mL water. 400 μ L Plasma + 200 ng practolol, add to SPE cartridge, wash twice with 1 mL water, wash with 1 mL acetone, allow to go dry. Elute with three 200 μ L aliquots of eluting solvent, combine the fractions, evaporate under nitrogen, suspend in 80 μ L mobile phase, inject a 50 μ L aliquot. (Eluting solvent was 10 mM acetic acid and 50 mM triethylamine in MeOH.)

HPLC VARIABLES

Guard column: Brownlee cyano

Column: 250 \times 4.6 6 μ m Zorbax CN

Mobile phase: MeCN:12.5 mM (NH₄)H₂PO₄:triethylamine 4:96:0.25, pH adjusted to 5.5 with 1.0 M phosphoric acid

Flow rate: 1.5

Injection volume: 50

Detector: UV 224

CHROMATOGRAM

Retention time: 6

Internal standard: practolol (9)

Limit of detection: < 10 ng/mL

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Simultaneous: disopyramide, lidocaine, metoprolol, nadolol, procainamide, quinidine, timolol, verapamil

KEY WORDS

plasma; SPE

REFERENCE

Verghese, C.; McLeod, A.; Shand, D. Rapid high-performance liquid chromatographic method for the measurement of atenolol in plasma using UV detection. *J.Chromatogr.*, **1983**, *275*, 367–375

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 100 mg Baxter C18 SPE cartridge with one volume MeOH and two volumes water. Dilute 10 μ L urine to 500 μ L with water. 500 μ L Serum or diluted urine + 50 μ L 5 μ g/mL albuterol in water, vortex for 30 s, add to SPE cartridge, wash with three 200 μ L aliquots of water, elute with two 500 μ L aliquots of MeOH. Evaporate the eluates to dryness under a stream of air at 40–45°, reconstitute the residue in 150 μ L water, vortex for 30 s, centrifuge at 14000 g for 4 min, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 5 μ m Adsorbosphere C-18

Column: 250 \times 4.6 5 μ m Adsorbosphere C-18

Mobile phase: MeCN:buffer 7:93 adjusted to pH 3.0 with 85% phosphoric acid (Buffer was 25 mM (NH₄)H₂PO₄ and 1 mM N,N-dimethyloctylamine.)

Flow rate: 1.5
Injection volume: 50
Detector: UV 224; F ex 228 em 310

CHROMATOGRAM

Retention time: 10.4
Internal standard: albuterol (7.1)
Limit of quantitation: 50 ng/mL (F, UV)

KEY WORDS

pharmacokinetics; SPE; serum

REFERENCE

Chatterjee, D.J.; Li, W.Y.; Hurst, A.K.; Koda, R.T. High-performance liquid chromatographic method for determination of atenolol from human plasma and urine: Simultaneous fluorescence and ultraviolet detection. *J.Liq.Chromatogr.*, **1995**, *18*, 791–806

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with MeOH and water. Hydrolyze 900 μ L serum with β -glucuronidase (EC 3.2.1.31 type H-1 from *Helix pomatia*) at 60° for 1 h, add 500 μ L (?) 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 μ L Urine + 500 μ L MeOH, filter, inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: 10 \times 4.6 5 μ m HP C18

Column: 150 \times 4.6 5 μ m 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: 2.5

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, 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 beta-adrenergic blocking agents from human biological fluids. *J.Chromatogr.A*, **1995**, *717*, 211–217

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma or 0.1 mL urine + 100 μ L 1 M pH 12 sodium phosphate buffer + 100 μ L 1 M NaOH + 10 mL dichloromethane:heptafluorobutanol 97:3, extract. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** Partisil C8**Mobile phase:** MeCN:MeOH:buffer 3:3:94 (Buffer was 10 mM ammonium phosphate buffer containing 11.6 mM phosphoric acid, pH 2.9.)**Detector:** F ex 220 em 200 (no cut-off filter)

CHROMATOGRAM**Limit of quantitation:** 5 ng/mL (plasma); 1000 ng/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCESowinski, K.M.; Forrest, A.; Wilton, J.H.; Taylor, A.M., II; Wilson, M.F.; Kazierad, D.J. Effect of aging on atenolol pharmacokinetics and pharmacodynamics. *J.Clin.Pharmacol.*, **1995**, *35*, 807-814

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 1 mL Plasma + 50 μ L 10 μ g/mL methoxamine in water + 100 μ L 1 M NaOH + 4 mL ethyl acetate, vortex for 30 s, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μ L saturated sodium carbonate and 200 μ L 187 mM (-)-menthyl chloroformate in MeCN, vortex for 30 s, add 1 mL water, add 2 mL chloroform, vortex for 30 s, centrifuge for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μ L mobile phase, vortex for 5 s, centrifuge for 5 min, inject a 20-60 μ L aliquot of the supernatant. Urine. Dilute urine 10 times with water. 100 μ L Diluted urine + 50 μ L 10 μ g/mL methoxamine in water + 100 μ L saturated sodium carbonate + 200 μ L 187 mM (-)-menthyl chloroformate in MeCN, vortex for 30 s, add 1 mL water, add 2 mL chloroform, vortex for 30 s, centrifuge for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μ L mobile phase, vortex for 5 s, centrifuge for 5 min, inject a 20-60 μ L aliquot of the supernatant.

HPLC VARIABLES**Guard column:** 50 mm long pellicular ODS (Whatman)**Column:** 100 \times 4.6 5 μ m Partisil 5 ODS3**Mobile phase:** MeCN:MeOH:water 35:22:43**Flow rate:** 1.2**Injection volume:** 20-60**Detector:** F ex 195 em no emission filter

CHROMATOGRAM**Retention time:** 13, 15 (enantiomers)**Internal standard:** methoxamine (18 (-), 20 (+))**Limit of detection:** 2.5 ng/mL

KEY WORDS

plasma; derivatization; pharmacokinetics

REFERENCEMehvar, R. Liquid chromatographic analysis of atenolol enantiomers in human plasma and urine. *J.Pharm.Sci.*, **1989**, *78*, 1035-1039

SAMPLE**Matrix:** bulk

Sample preparation: Dissolve 10 μmol 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 μmol 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°, $[\alpha]_{546} = -133^\circ$ (c=1) in MeCN).

HPLC VARIABLES

Column: 125 \times 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: formulations

Sample preparation: Dissolve 2 mg tablet or capsule in 10 mL pH 10 solution, extract twice with 2 mL ether, combine extracts, filter, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 μm β -cyclodextrin bonded C18 (Advanced Separation Technologies)

Mobile phase: MeCN:MeOH:acetic acid:triethylamine 95:5:0.3:0.2

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 33, 37 (enantiomers)

OTHER SUBSTANCES

Simultaneous: metoprolol, propranolol

KEY WORDS

capsules; tablets; chiral

REFERENCE

Tran, C.D.; Dotlich, M. Enantiomeric separation of beta-blockers by high performance liquid chromatography. *J.Chem.Educ.*, **1995**, *72*, 71–73

SAMPLE

Matrix: formulations

Sample preparation: Dilute 500 μL to 10 mL with water. 100 μL Diluted sample + 100 μL 2.5 $\mu\text{g}/\text{mL}$ sotalol + 100 μL saturated sodium tetraborate adjusted to pH 9 with HCl + 500 μL water + 5 mL dichloromethane:isopropanol 3:1, agitate on mechanical shaker for 5 min, centrifuge at 800 g for 3 min. Evaporate organic layer to dryness at 45° under a stream of nitrogen. Dissolve residue in 200 μL 50 mM sulfuric acid, mix for 30 s, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: Novapak C18 guard insert

Column: 100 \times 5 Novapak C18

Mobile phase: MeCN:10 mM potassium phosphate buffer adjusted to pH 3.2 with 0.2 M phosphoric acid 20:80 containing 3 mM 1-octanesulfonic acid

Flow rate: 1

Injection volume: 30

Detector: UV 226

CHROMATOGRAM

Retention time: 3.5

Internal standard: sotalol (5)

KEY WORDS

stability indicating; oral liquid

REFERENCE

Garner, S.S.; Wiest, D.B.; Reynolds, E.R., Jr. Stability of atenolol in an extemporaneously compounded oral liquid. *Am.J.Hosp.Pharm.*, **1994**, *51*, 508–511

SAMPLE

Matrix: perfusate

Sample preparation: 50 μL Perfusate + 50 μL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 μL 30 $\mu\text{g}/\text{mL}$ salicylic acid in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 Cosmosil 5C18-P (Nacalai Tesque)

Mobile phase: MeOH:50 mM NaH_2PO_4 20:80

Flow rate: 1

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Internal standard: salicylic acid

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 beta-blockers. *Pharm.Res.*, **1995**, *12*, 1146–1150

SAMPLE**Matrix:** perfusate**Sample preparation:** Add perfusate to an equal volume of MeOH, vortex, centrifuge, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 Nucleosil 5C18**Mobile phase:** MeCN:1% phosphoric acid 60:40 containing 2.5 mM sodium dodecyl sulfate**Detector:** F ex 280 em 333

REFERENCE

Kobayashi, D.; Matsuzawa, T.; Sugibayashi, K.; Morimoto, Y.; Kobayashi, M.; Kimura, M. Feasibility of use of several cardiovascular agents in transdermal therapeutic systems with *l*-menthol-ethanol system on hairless rat and human skin. *Biol.Pharm.Bull.*, **1993**, *16*, 254–258

SAMPLE**Matrix:** saliva**Sample preparation:** Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. 1 mL Supernatant + 50 µL 100 µg/mL tertatolol, add to the SPE cartridge, wash with 500 µL water, wash with 500 µL MeCN, elute with two 500 µL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 µL mobile phase, mix for 15 s, inject a 40 µL aliquot. (Acidified MeOH was 50 mL MeOH + 300 µL 96% acetic acid.)

HPLC VARIABLES**Guard column:** RCSS silica guard-pack (Waters)**Column:** 250 × 4.6 Chiralcel OD-H**Mobile phase:** n-Hexane:EtOH:diethylamine 50:50:1**Flow rate:** 1**Injection volume:** 40**Detector:** F ex 225 em 290 cut-off filter

CHROMATOGRAM**Internal standard:** (R, S)-tertatolol

KEY WORDS

SPE; chiral

REFERENCE

Höld, K.M.; de Boer, D.; Zuidema, J.; Maes, R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva. *J.Chromatogr.B*, **1995**, *663*, 103–110

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 \times 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 \times 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

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-C₁ 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 KH₂PO₄ 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: Inject a 40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil MOS C-8

Mobile phase: MeOH:water 70:30 containing 0.02% dimethyloctylamine, 25 mM sodium hexanesulfonate, and 20 mM acetic acid

Flow rate: 1
Injection volume: 40
Detector: F ex 275 em 305

CHROMATOGRAM

Retention time: 3.3

OTHER SUBSTANCES

Simultaneous: alprenolol, pindolol, propranolol (UV 288)

REFERENCE

Adson, A.; Burton, P.S.; Raub, T.J.; Barsuhn, C.L.; Audus, K.L.; Ho, N.F.H. Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: Uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers. *J.Pharm.Sci.*, **1995**, *84*, 1197–1204

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot of a 1 mg/mL solution.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m 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' 0.94, 1.75 (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

Sample preparation: Inject an aliquot of a 200 μ M solution in MeOH.

HPLC VARIABLES

Column: 100 \times 4.7 7 μ m Hypercarb (Shandon)

Mobile phase: MeOH containing 5 mM N-benzoyloxycarbonylglycyl-L-proline and 4.5 mM NaOH

Column temperature: 17

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: k' 13 (first enantiomer)

KEY WORDS

chiral; α = 1.09

REFERENCE

Huynh, N.-H.; Karlsson, A.; Pettersson, C. Enantiomeric separation of basic drugs using N-benzyloxy-carbonylglycyl-L-proline as counter ion in methanol. *J.Chromatogr.A*, **1995**, *705*, 275–287

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, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinarizine, 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, tripeleppamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kalishan, R.; Nasal, A.; Turowski, M. Binding site for basic drugs on α₁-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: 5.33 (A), 3.11 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, mocllobemide, 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

some 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 × 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' 9.78

KEY WORDS

chiral; α 1.14

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: 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, 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, dapson, 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, fenpropfen, 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, isocarboxystyryl, 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, mepheryptoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldridin, 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, pyrillamine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triproilidine, 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: 50 μ L Solution + 50 μ L pH 7.4 PBS + 100 μ L 30 μ g/mL salicylic acid in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 Cosmosil 5C18-P (Nacalai Tesque)

Mobile phase: MeOH:50 mM NaH_2PO_4 20:80

Flow rate: 1

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Internal standard: salicylic acid

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: Prepare a 25 μ g/mL solution in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 3 μ m 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: 6.55

OTHER SUBSTANCES

Also analyzed: clonidine, 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

Sample preparation: Filter (0.22 μ m), inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 13.6

OTHER SUBSTANCES

Simultaneous: acebutolol, alprenolol, carteolol, metoprolol, oxprenolol, pindolol

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: 250 \times 4.6 cellulose tris(3,5-dimethylphenylcarbamate)

Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1

Flow rate: 0.5

Detector: UV

CHROMATOGRAM

Retention time: k' 3.54 (of first (+) enantiomer)

KEY WORDS

chiral; α 1.58

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 10 $\mu\text{g}/\text{mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 \times 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: 2.03

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, azacyclonal, amethane, benactyzine, benperidol, benzethidine, benzocaine, benzocamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butetha-

mate, 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, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdiazene, 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, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, 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: tablets

Sample preparation: Grind tablet equivalent to about 50 mg nadolol, add 200 mL mobile phase, sonicate for 15 min, make up to 250 mL with mobile phase, filter or centrifuge, to 20 mL solution add 5 mL 1.2 mg/mL atenolol in mobile phase, mix, 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: 4.5

Internal standard: atenolol

OTHER SUBSTANCES

Simultaneous: acebutolol, alprenolol, metoprolol, nadolol, oxprenolol, pindolol, practolol, propranolol, sotalol, timolol

KEY WORDS

stability-indicating; atenolol is IS

REFERENCE

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

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 μ m 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: 7

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, amphetamine, 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

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Bailey, C.J.; Ruane, R.J.; Wilson, I.D. Packed-column supercritical fluid chromatography of β -blockers. *J.Chromatogr.Sci.*, **1994**, *32*, 426–429 [SFC; also alprenolol, labetalol, metoprolol, oxprenolol, pindolol, practolol, propranolol, toliprolol, xamoterol]

- Hamoir, T.; Massart, D.L. Retention prediction for β -adrenergic blocking drugs in normal-phase liquid chromatography. *J.Chromatogr.A*, **1994**, *673*, 1–10 [column temp 30; cyanopropyl column; also acebutolol, alprenolol, bunitrolol, bupranolol, carazolol, mepindolol, metipranolol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, practolol, prenalterol, propranolol, tertatolol]
- Hermansson, J.; Grahn, A. Resolution of racemic drugs on a new chiral column based on silica-immobilized cellobiohydrolase. Characterization of the basic properties of the column. *J.Chromatogr.*, **1994**, *687*, 45–59 [chiral; also acebutolol, betaxolol, bisoprolol, carbutoleol, cathinone, cimetidine, dobutamine, dopropizine, epanolol, epinephrine, laudanosine, metanephrine, metoprolol, moprolol, norepinephrine, normetanephrine, octopamine, oxybutynine, pamatolol, practolol, prilocaine, propafenone, proxiphylline, sotalol, talinolol, tetrahydropapaveroline, tetramisole, timolol, tolamolol, toliprolol]
- Kobayashi, D.; Matsuzawa, T.; Sugibayashi, K.; Morimoto, Y.; Kimura, M. Analysis of the combined effect of 1-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. *Pharm.Res.*, **1994**, *11*, 96–103 [also morphine, naloxone, nifedipine, nitrendipine, vinpocetine]
- Egginger, G.; Lindner, W.; Kahr, S.; Stoschitzky, K. Stereoselective HPLC bioanalysis of atenolol enantiomers in plasma: application to a comparative human pharmacokinetic study. *Chirality*, **1993**, *5*, 505–512
- Josefsson, M.; Carlsson, B.; Norlander, B. Fast chromatographic separation of (–)-menthyl chloroformate derivatives of some chiral drugs, with special reference to amlodipine, on porous graphitic carbon. *Chromatographia*, **1993**, *37*, 129–132 [derivatization; chiral; also amlodipine, mexiletine, propranolol, sotalol]
- Armstrong, D.W.; Chen, S.; Chang, C.; Chang, S. A new approach for the direct resolution of racemic beta adrenergic blocking agents by HPLC. *J.Liq.Chromatogr.*, **1992**, *15*, 545–556 [chiral; also alprenolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol, propranolol, timolol]
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- Shen, J.; Wanwimolruk, S.; Hung, C.T.; Zoest, A.R. Quantitative analysis of β -blockers in human plasma by reversed-phase ion-pair high-performance liquid chromatography using a microbore column. *J.Liq.Chromatogr.*, **1991**, *14*, 777–793 [plasma; microbore; fluorescence detection; UV detection; LOD 1–10 ng/mL; also labetalol, metoprolol, pindolol, propranolol; oxprenolol (IS)]
- Teitelbaum, Z.; Ben-Dom, N.; Terry, S. A liquid chromatographic method for the determination of atenolol in human plasma. *J.Liq.Chromatogr.*, **1991**, *14*, 3735–3744 [plasma; metoprolol (IS); LOD 12.6 ng/mL; fluorescence detection]
- Alebic-Kolbah, T.; Plavsic, F.; Wolf-Coporda, A. Determination of serum atenolol using HPLC with fluorescence detection following isolation with activated charcoal. *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1777–1781
- Bui, K.H.; French, S.B. Direct serum injection and analysis of drugs with aqueous mobile phases containing triethylammonium acetate. *J.Liq.Chromatogr.*, **1989**, *12*, 861–873 [serum; plasma; dog; rat; fluorescence detection; UV detection; also antipyrine, hexaphenone, metoprolol, naproxen, propranolol]
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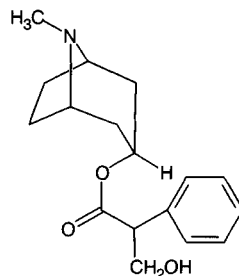
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- Wilson, M.J.; Ballard, K.D.; Walle, T. Preparative resolution of the enantiomers of the beta-blocking drug atenolol by chiral derivatization and high performance liquid chromatography. *J.Chromatogr.*, **1988**, *431*, 222–227
- Buhring, K.U.; Garbe, A. Determination of the new beta-blocker bisoprolol and of metoprolol, atenolol and propranolol in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *382*, 215–224
- Miller, L.G.; Greenblatt, D.J. Determination of atenolol in plasma by high-performance liquid chromatography with application to single-dose pharmacokinetics. *J.Chromatogr.*, **1986**, *381*, 201–204
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Atropine

Molecular formula: C₁₇H₂₃NO₃

Molecular weight: 289.4

CAS Registry No.: 51-55-8 (atropine), 52-88-0 (atropine methylnitrate), 101-31-5 (hyoscyamine), 306-03-6 (hyoscyamine hydrobromide), 6835-16-1 (hyoscyamine sulfate dihydrate), 620-61-1 (hyoscyamine sulfate)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 5 ng/mL scopolamine in MeOH, vortex briefly, add 50 μ L 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 2 5 μ m BDS C18 (Keystone)

Column: 50 \times 3 3 μ m 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

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 μ L 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 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:** 4.3**Limit of detection:** 0.5-2.5 ng/mL

OTHER SUBSTANCES**Simultaneous:** atipamezole, butorphanol, flumazenil, ketamine, medetomidine, midazolam, xylazine

KEY WORDS

plasma; SPE; dog

REFERENCEKanazawa, 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:** blood**Sample preparation:** 2 mL Plasma + 100 μ L 1 M NaOH + 10 mL chloroform, shake for 30 s, remove a 9 mL aliquot of the organic phase and add it to 1 mL 100 mM HCl, extract. Remove a 900 μ L aliquot of the aqueous layer and add it to 100 μ L 5 M NaOH, heat at 38° for 3 h, acidify with 5 M HCl, add 8 mL dichloromethane, vortex for 2 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40-45°, add 3 mg solid potassium bicarbonate, add 100 μ L 10 μ g/mL mandelic acid in MeCN, add 300 μ L 50 μ g/mL 4-bromomethyl-7-methoxycoumarin in MeCN, add 100 μ L 15 μ g/mL 18-crown-6 in MeCN, vortex for 15 s, heat at 70° for 45 min, inject a 25 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m ODS (Dupont)**Mobile phase:** MeCN:buffer 33:67 (Buffer was 10 mM (NH₄)H₂PO₄ adjusted to pH 5.0.)**Column temperature:** 40**Flow rate:** 2**Injection volume:** 25**Detector:** F ex 328 em 389 (cutoff filter)

CHROMATOGRAM**Retention time:** 10**Internal standard:** mandelic acid (7)**Limit of detection:** 108 ng/mL**Limit of quantitation:** 125 ng/mL

KEY WORDS

plasma; derivatization; atropine determined after hydrolysis to tropic acid

REFERENCELi, S.; Wahba Khalil, S.K. An HPLC method for determination of atropine in human plasma. *J.Liq.Chromatogr.*, **1990**, *13*, 1339-1350

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 μ L 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 hyosecyamine and scopolamine in solanaceous crude drugs. *Chem.Pharm.Bull.*, **1989**, *37*, 2456–2458

SAMPLE**Matrix:** formulations**Sample preparation:** Injections and ophthalmic solutions. Dilute with water to an atropine concentration of 80 μg/mL, inject a 20 μL aliquot. Ointment. Weigh out ointment equivalent to about 4 mg atropine sulfate, add 10 mL THF:water 80:20, sonicate and swirl until the ointment is completely dispersed, make up to 50 mL with water, filter (0.45 μm), inject a 20 μL aliquot

HPLC VARIABLES**Column:** 250 × 4.6 μm Spherisorb CN**Mobile phase:** MeCN:50 mM NaH₂PO₄ 10:90, pH adjusted to 4.0 with 10% phosphoric acid**Flow rate:** 1**Injection volume:** 20**Detector:** UV 220

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES**Simultaneous:** phenol, tropic acid**Noninterfering:** benzalkonium chloride, benzyl alcohol, chlorobutanol, methylparaben

KEY WORDSinjections; ophthalmic solutions; ointments

REFERENCE

Lehr, G.J.; Yuen, S.M.; Lawrence, G.D. Liquid chromatographic determination of atropine in nerve gas antidotes and other dosage forms. *J.AOAC Int.*, **1995**, *78*, 339–343

SAMPLE**Matrix:** formulations**Sample preparation:** Inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 100 × 4.6 Spheri-5 RP-8

Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 1 M KOH.)

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 7.8

Limit of detection: 8.6 $\mu\text{g/mL}$

OTHER SUBSTANCES

Simultaneous: ondansetron

Noninterfering: degradation products

KEY WORDS

injections; saline

REFERENCE

Venkateshwaran, T.G.; King, D.T.; Stewart, J.T. HPLC determination of ondansetron-atropine and ondansetron-glycopyrrolate mixtures in 0.9% sodium chloride injection. *J.Liq.Chromatogr.*, **1995**, *18*, 2647–2659

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 12.5 \times 4 5 μm Zorbax RX-C18

Column: 250 \times 4.6 5 μm Zorbax RX-C18

Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM NaH_2PO_4 + 1 mM tetramethylammonium chloride + 0.5 mM 1-octanesulfonic acid adjusted to pH 3.5 with concentrated orthophosphoric acid.)

Column temperature: 25

Flow rate: 1

Injection volume: 20

Detector: UV 203

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: HI-6, obidoxime, phenol, tropic acid

Also analyzed: pralidoxime chloride

KEY WORDS

nerve agent antidote mixtures

REFERENCE

Paddle, B.M.; Dowling, M.H. Simple high-performance liquid chromatographic method for assessing the deterioration of atropine-oxime mixtures employed as antidotes in the treatment of nerve agent poisoning. *J.Chromatogr.*, **1993**, *648*, 373–380

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Whatman PXS ODS-3 C18

Mobile phase: MeCN:Buffer 25:75 (Buffer was 1.08 g sodium octanesulfonate in 900 mL of water adjusted to pH 3.5 with glacial acetic acid and diluted to 1 L with water.)

Flow rate: 2

Injection volume: 20

Detector: UV 229

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: milrinone

KEY WORDS

injections; 10% calcium chloride; 7.5% sodium bicarbonate; stability-indicating

REFERENCE

Wilson, T.D.; Forde, M.D. Stability of milrinone and epinephrine, atropine sulfate, lidocaine hydrochloride, or morphine sulfate injection. *Am.J.Hosp.Pharm.*, **1990**, *47*, 2504–2507

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Crush tablet, add 2 mL water, add 500 μL 0.1 M NaOH, if not basic to litmus add 0.1 M NaOH dropwise until it is. Extract with 10 mL chloroform then with three 5 mL portions of chloroform. Pass all extracts through a sodium sulfate column and then wash column with 2 mL chloroform. Evaporate all organic extracts with heating under a stream of air. Take up residue in 1 mL MeOH, inject a 100 μL aliquot. Injections. Evaporate 2 mL to dryness on a steam bath, take up residue in 5 mL water, add 500 μL 0.1 M NaOH, if not basic to litmus add 0.1 M NaOH dropwise until it is. Extract with 10 mL chloroform then with three 5 mL portions of chloroform. Pass all extracts through a sodium sulfate column and then wash column with 2 mL chloroform. Evaporate all organic extracts with heating under a stream of air. Take up residue in 2 mL MeOH, inject a 100 μL aliquot. (Sodium sulfate column was a 300 × 20 glass chromatography column containing 10 g anhydrous sodium sulfate washed with 10 mL chloroform before use.)

HPLC VARIABLES

Column: 250 × 4.6 Alltech C18

Mobile phase: MeOH:20 g/L sodium pentanesulfonate 95:5

Flow rate: 1

Injection volume: 100

Detector: F ex 255 em 285

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: scopolamine

Noninterfering: ergotamine, phenobarbital

KEY WORDS

tablets; injections

REFERENCE

Cieri, U.R. Determination of small quantities of atropine in commercial preparations by liquid chromatography with fluorescence detection. *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 1042–1045

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute 10 mL eye drops to 100 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 3.9 5 μ m Perkin-Elmer C18**Mobile phase:** MeOH:water:heptanesulfonic acid solution 500:500:25 (Heptanesulfonic acid solution was 5 g heptanesulfonic acid + 20 mL glacial acetic acid diluted to 150 mL with water.)**Flow rate:** 1.8**Injection volume:** 20**Detector:** UV 225

CHROMATOGRAM**Retention time:** 4**Limit of detection:** 5 μ g/mL

OTHER SUBSTANCES**Simultaneous:** tropic acid

KEY WORDS

eye drops

REFERENCERichard, A.; Andermann, G. Simultaneous determination of atropine sulphate and tropic acid by reversed phase high-pressure liquid chromatography. *Pharmazie*, **1984**, *39*, 866–867

SAMPLE**Matrix:** formulations**Sample preparation:** Tablets, capsules. Powder tablets or remove contents of capsules, weigh out amount equivalent to about 600 μ g 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 μ g/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 μ L 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 μ L MeOH, evaporate to dryness with a stream of air at 40°, dissolve the residue in 300 μ L water, inject a 20 μ L aliquot. Elixirs. Add an amount equivalent to about 600 μ g 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 μ g/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 μ L 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 μ L MeOH, evaporate to dryness with a stream of air at 40°, dissolve the residue in 300 μ L water, inject a 20 μ L 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: hyoscyamine, phenobarbital, scopolamine

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: 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: 50 μg/g

OTHER SUBSTANCES

Simultaneous: tropic acid, scopolamine

KEY WORDS

freeze-dried plant leaves

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:** 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

REFERENCEHagemann, 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:** Dissolve plant extract 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 30:70**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

REFERENCEAuriola, 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:** solutions

HPLC VARIABLES

Column: 250 × 4.1 6 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: 2.7

OTHER SUBSTANCES

Simultaneous: barbituric acid, codeine, diphenhydramine, 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 × 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)₃²⁺ in 50 mM sodium sulfate (continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)₃³⁺ 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 pumped at 0.3 mL/min mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

CHROMATOGRAM

Retention time: 3

Limit of detection: 0.1-1 μg/mL

OTHER SUBSTANCES

Simultaneous: cyclobenzaprine, cyclopentolate, dicyclomine, procyclidine

KEY WORDS

post-column reaction

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

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: 6.05 (A), 3.75 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, 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, fenopropfen, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazo-line, 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, protripty-line, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyra-zone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethyl-perazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, tri-methoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 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, 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, dapson, 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, fenpropfen, 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, isocarboxtyril, isocarboxamid, 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, methylidopa, methylidopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylihdrin, 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, pyrillamine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thibendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triproilidine, 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 cellulose tris(3,5-dimethylphenylcarbamate)

Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1

Flow rate: 0.5

Detector: UV

CHROMATOGRAM

Retention time: k' 0.72 (of first (+) enantiomer)

KEY WORDS

chiral; α 1.62

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: Dissolve in MeOH or water to 0.1%, inject an aliquot

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 2:98

Flow rate: 0.5

Injection volume: 1

Detector: UV

CHROMATOGRAM

Retention time: k' 6.83 (d-isomer)

KEY WORDS

chiral; optical isomers are separated

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: 300 × 3.9 10 μ m 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: 13

OTHER SUBSTANCES

Simultaneous: codeine, dansylamide, dansylcadaverine, doxorubicin, 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: 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 230

CHROMATOGRAM

Retention time: 17

OTHER SUBSTANCES

Simultaneous: homatropine, methscopolamine, tropic acid, atropine methyl, scopolamine

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

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Theodoridis, G.; Papadoyannis, I.; Vasiliakiotis, G.; Tsoukali-Papadopoulou, H. Reversed-phase high-performance liquid chromatography–photodiode-array analysis of alkaloid drugs of forensic interest. *J.Chromatogr.B*, **1995**, *668*, 253–263 [also amphetamine, bamifylline, caffeine, cocaine, codeine, diamorphine, ethylmorphine, flufenamic acid, methadone, morphine, nalorphine, norcodeine, papaverine, quinine, scopolamine, strychnine, theobromine, theophylline, tolfenamic acid]

Buch, U.; Isenberg, E.; Buch, H.P. HPLC assay for atropine in serum and protein solutions after in vitro addition of the tropane alkaloid. *Methods Find.Exp.Clin.Pharmacol.*, **1994**, *16*, 361–365

Pohjola, J.; Harpf, M. Determination of atropine and obidoxime in automatic injection devices used as antidotes against nerve agent intoxication. *J.Chromatogr.*, **1994**, *686*, 350–354 [simultaneous obidoxime; phenol (IS)]

Schill, G.; Wainer, I.W.; Barkan, S.A. Chiral separation of cationic drugs on an α1-acid glycoprotein bonded stationary phase. *J.Liq.Chromatogr.*, **1986**, *9*, 641–666 [chiral; also bromdiphenhydramine, brompheniramine, bupivacaine, butorphanol, carbinoxamine, chlorpheniramine, clidinium, cocaine, cyclopentolate, dimethindene, diperidone, disopyramide, doxylamine, ephedrine, homatropine, labetalol B, labetalol, labetalol A, mepensolate, mepivacaine, methadone, methorphan, methylatropine, methylhomatropine, methylphenidate, metoprolol, nadolol, nadolol A, nadolol B, oxprenolol, oxypheyclimine, phenmetrazine, phenoxybenzamine, promethazine, pronethalol, propoxyphene, propranolol, pseudoephedrine, terbutaline, tocinamide, tridihexethyl]

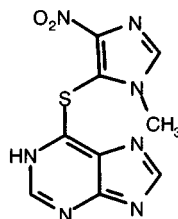
Achari, R.G.; Jacob, J.T. A study of the retention behavior of some basic drug substances by ion-pair HPLC. *J.Liq.Chromatogr.*, **1980**, *3*, 81–92 [also N-acetylprocainamide, antazoline, caffeine, chlorpheniramine, codeine, ephedrine, epinephrine, naphazoline, papaverine, pheniramine, phenylephrine, phenylpropanolamine, procainamide, quinidine, scopolamine, xylocaine]

Azathioprine

Molecular formula: C₉H₇N₇O₂S

Molecular weight: 277.3

CAS Registry No.: 446-86-6



SAMPLE

Matrix: blood

Sample preparation: 500 μ L 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 μ L mobile phase, vortex for 10 s, sonicate for 10 min, inject a 200 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4.6 5 μ m LiChrospher 100 RP 18

Column: 250 \times 4.6 5 μ m 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 μ L methyl iodide to a solution of 220 mg azathioprine in 7 mL DMF at 0-5°, stir under nitrogen for 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.; Wellhömer, H.H. High-performance liquid chromatographic assay for the measurement of azathioprine in human serum samples. *J. Chromatogr. B*, **1996**, 675, 287-294

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Isolute C8 SPE cartridge (International Sorbent Technology) with 2.5 mL MeOH and 3.5 mL 10 mM pH 7.0 phosphate buffer. 1 mL Plasma + 50 μ L 1.5 μ g/mL guaneran + 2 mL 10 mM pH 7.0 phosphate buffer, mix, add to the SPE cartridge at 2.5 mL/min, wash with 3 mL MeCN:pH 7.0 phosphate buffer 1:99, air dry for 2 min, elute with 2 mL MeOH:ethyl acetate 5:95. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 80 × 4.6 3 μm HSpecosphere 3CR C8 (Perkin-Elmer)**Mobile phase:** MeCN:10 mM pH 6.2 sodium phosphate buffer 9:91**Flow rate:** 1.2**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 8.9**Internal standard:** guaneran (6-[(1-methyl-4-nitro-5-imidazolyl)thio]-2-aminopurine, Wellcome Foundation) (7.7)**Limit of detection:** 0.5 ng/mL

OTHER SUBSTANCES**Simultaneous:** caffeine**Noninterfering:** aspirin, chloroquine, cyclosporin, diltiazem, nifedipine, prednisolone

KEY WORDSplasma; pharmacokinetics; SPE

REFERENCEAlbertioni, F.; Pettersson, B.; Ohlman, S.; Peterson, C. Analysis of azathioprine and 6-mercaptopurine in plasma in renal transplant recipients after administration with oral azathioprine. *J.Liq. Chromatogr.*, **1995**, *18*, 3991–4005

SAMPLE**Matrix:** blood**Sample preparation:** Condition a 1 mL Sep-Pak silica SPE cartridge with 3 mL ethyl acetate and vacuum dry for 1 min. 1 mL Plasma + 2.8 μg antipyrine, add to SPE cartridge, wash with 5 mL benzene (Caution! Benzene is a carcinogen!) or hexane over 1 min, vacuum dry for 1 min, elute with 5 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, sonicate, inject all of the sample.

HPLC VARIABLES**Guard column:** C18 Guard-Pak (Waters)**Column:** 100 × 8 4 μm 8 NV C18 Radial-Pak (Waters)**Mobile phase:** MeOH:10 mM pH 4.5 sodium phosphate 13:87**Flow rate:** 3**Injection volume:** 200**Detector:** UV 280

CHROMATOGRAM**Retention time:** 7**Internal standard:** antipyrine (17)**Limit of quantitation:** 5 ng/mL

OTHER SUBSTANCES**Simultaneous:** acetaminophen, aspirin, carmustine, chlorambucil, cytarabine, dacarbazine, diclofenac, etoposide, 5-fluorouracil, ifosfamide, indomethacin, lomustine, methotrexate, naproxen, salicylic acid, tegafur, teniposide, thioguanine**Noninterfering:** betamethasone, carboplatin, cyclophosphamide, cyclosporine A, ibuprofen, thiotepa

KEY WORDS

plasma; rabbit; SPE; human; pharmacokinetics

REFERENCE

el-Yazigi, A.; Abdel Wahab, F. Expedient liquid chromatographic analysis of azathioprine in plasma by use of silica solid phase extraction. *Ther. Drug Monit.*, **1992**, *14*, 312–316

SAMPLE

Matrix: blood

Sample preparation: 200 μL Serum + 5 μL 50 $\mu\text{g}/\text{mL}$ 2-ethyl-4-oxoquinazoline in EtOH + 100 μL reagent, let stand at room temperature for 1 h, add 1.8 mL ethyl acetate, mix, centrifuge at 1800 g for 5 min, remove 1.5 mL of the supernatant, repeat the extraction. Combine the organic layers and evaporate them to dryness under reduced pressure below 30°, reconstitute the residue in 100 μL initial mobile phase, inject a 90 μL aliquot. (Reagent was 30 mg N-ethylmaleimide in 2 mL 50 mM pH 7.0 phosphate buffer, prepare fresh daily.)

HPLC VARIABLES

Column: 10 μm $\mu\text{Bondapak C18}$

Mobile phase: Gradient. MeCN:10 mM KH_2PO_4 9:91 for 26 min, then 50:50 for 1 min (step gradient).

Flow rate: 1.5

Injection volume: 90

Detector: UV 280

CHROMATOGRAM

Retention time: 13.4

Internal standard: 2-ethyl-4-oxoquinazoline (28)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: 6-mercaptopurine

KEY WORDS

serum

REFERENCE

Tsutsumi, K.; Otsuki, Y.; Kinoshita, T. Simultaneous determination of azathioprine and 6-mercaptopurine in serum by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, **1982**, *231*, 393–399

SAMPLE

Matrix: formulations

Sample preparation: Dissolve crushed tablets or the freeze-dried compound for injection in 20 mM NaOH. Add a 10 mL aliquot of this solution (or a saline injection) to 10 mL 3 mg/mL theophylline in 20 mM NaOH, inject a 1.5 μL aliquot.

HPLC VARIABLES

Column: 100 \times 5 μm ODS-Hypersil

Mobile phase: MeOH:25 mM KH_2PO_4 :glacial acetic acid 20:79:5 adjusted to pH 4.50 (Flush column with MeOH:water 60:40 at the end of each day.)

Flow rate: 1.5

Injection volume: 1.5

Detector: UV 240

CHROMATOGRAM

Retention time: 4.2

Internal standard: theophylline (3.5)

OTHER SUBSTANCES

Simultaneous: impurities, degradation products, 6-mercaptopurine

KEY WORDS

stability-indicating; injections; tablets

REFERENCE

Fell, A.F.; Plag, S.M.; Neil, J.M. Stability-indicating assay for azathioprine and 6-mercaptopurine by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1979**, *186*, 691–704

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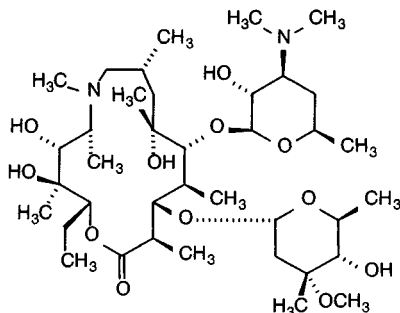
Ding, T.L.; Benet, L.Z. Determination of 6-mercaptopurine and azathioprine in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1979**, *163*, 281–288

Azithromycin

Molecular formula: C₃₈H₇₂N₂O₁₂

Molecular weight: 749.0

CAS Registry No.: 83905-01-5



SAMPLE

Matrix: blood

Sample preparation: 50 μ L Serum + 50 μ L 60 mM potassium carbonate + 50 μ L 50 ng/mL IS in MeCN water 50:50, mix, add 200 μ L water, vortex for several s, add 3 mL MTBE, vortex for 20 s, centrifuge at 3300 rpm for 3 min. Remove the organic layer and evaporate it to dryness in a vortex evaporator at 40°, reconstitute the residue in 100 μ L mobile phase, sonicate, vortex, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 30 \times 4.6 3 μ m Hypersil ODS

Mobile phase: MeCN:MeOH:THF:50 mM ammonium acetate 44:19:3:34

Flow rate: 1

Injection volume: 50

Detector: MS, SCIEX API III, atmospheric pressure ionization, nebulizer probe 450°, 2.5 μ A Corona discharge needle, quadrupole mass filter, 0.002 inch pinhole aperture, SIM m/z 749 and 752

CHROMATOGRAM

Retention time: 1.9

Internal standard: trideuteroazithromycin

Limit of quantitation: 10 ng/mL

KEY WORDS

serum

REFERENCE

Fouda, H.G.; Schneider, R.P. Quantitative determination of the antibiotic azithromycin in human serum by high-performance liquid chromatography (HPLC)-atmospheric pressure chemical ionization mass spectrometry: Correlation with a standard HPLC-electrochemical method. *Ther. Drug Monit.*, **1995**, *17*, 179-183

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 25 μ L 10 μ g/mL IS in MeCN + 1 mL 30 mM potassium carbonate, vortex, add 5 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 37°, reconstitute the residue in 300 μ L mobile phase, vortex for 30 s, add 1 volume hexane, vortex, centrifuge, remove the aqueous layer, inject a 50 μ L aliquot of the aqueous layer (*J. Chromatogr.* 1991, 565, 321).

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Nucleosil C18

Column: 125 \times 4.6 5 μ m Nucleosil C18

Mobile phase: MeCN:40 mM Na₂HPO₄:5 mM tetrabutylammonium phosphate 33:50:50, adjusted to pH 7.0 with 25% phosphoric acid

Flow rate: 1

Injection volume: 50

Detector: E, ESA 5100A Coulochem, guard cell +1 V, dual electrode analytical cell +0.7 and +0.8 V

CHROMATOGRAM

Retention time: 5.8

Internal standard: n-propyl analog of azithromycin (7.8)

Limit of quantitation: 8 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; pharmacokinetics

REFERENCE

Riedel, K.-D.; Wildfeuer, A.; Laufen, H.; Zimmermann, T. Equivalence of a high-performance liquid chromatographic assay and a bioassay of azithromycin in human serum samples. *J.Chromatogr.*, **1992**, *576*, 358-362

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 25 µL 10 µg/mL IS in MeCN + 1 mL 30 mM potassium carbonate, vortex, add 5 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 37°, reconstitute the residue in 300 µL mobile phase, vortex for 30 s, add 1 volume hexane, vortex, centrifuge, remove the aqueous layer, inject a 100 µL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 21 × 3 40 µm glass bead column (Waters)

Column: 50 × 4.6 5 µm Chromegabond alkylphenyl (ES Industries)

Mobile phase: MeCN:MeOH:20 mM ammonium acetate:20 mM sodium perchlorate 45:10:22:23, adjust apparent pH to 6.8-7.2 with glacial acetic acid

Flow rate: 1

Injection volume: 100

Detector: E, ESA 5100A Coulochem, ESA 5020 guard cell +1 V, ESA 5010 dual electrode analytical cell, screen electrode +0.7 V, detector electrode +0.8 V, porous carbon electrodes

CHROMATOGRAM

Retention time: 9

Internal standard: 9a-N-propyl analog of azithromycin (13)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; human; mouse; rat; dog; rabbit

REFERENCE

Shepard, R.M.; Duthu, G.S.; Ferraina, R.A.; Mullins, M.A. High-performance liquid chromatographic assay with electrochemical detection for azithromycin in serum and tissues. *J.Chromatogr.*, **1991**, *565*, 321-337

SAMPLE**Matrix:** blood, tissue**Sample preparation:** Serum. 500 μ L Serum + 25 μ L 2 μ g/mL IS in MeCN + 500 μ L 60 mM potassium carbonate, vortex, add 5 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 500 μ L 15 mM pH 3.1 citric acid, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the aqueous layer and add it to 1 mL 60 mM potassium carbonate, vortex for 1 min, add 5 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 37°, reconstitute the residue in 300 μ L MeCN:water 50:50, vortex for 30 s, add 1 volume hexane, vortex, centrifuge, remove the aqueous layer, inject a 60 μ L aliquot of the aqueous layer. (For high azithromycin concentrations extraction into citric acid and back extraction is not necessary.) Tissue. 1 g Tissue + 9 volumes MeCN + 50 μ L 20 μ g/mL IS in MeOH, homogenize (Polytron PT 10/35) for 10 s, centrifuge at 2000 g for 10 min. Remove a 500 μ L aliquot of the supernatant and evaporate it to dryness under vacuum at 50°, reconstitute the residue 500 μ L 60 mM potassium carbonate, add 5 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 37°, reconstitute the residue in 300 μ L MeCN:water 50:50, vortex for 30 s, add 1 volume hexane, vortex, centrifuge, remove the aqueous layer, inject a 20-60 μ L aliquot of the aqueous layer.

HPLC VARIABLES**Guard column:** 21 \times 3 40 μ m glass bead column (Waters)**Column:** 150 \times 4.6 5 μ m Chromegabond γ -RP-1 alumina (ES Industries)**Mobile phase:** MeCN:50 mM potassium phosphate 30:70, adjusted to an apparent pH of 11.0 with 1 M KOH**Flow rate:** 1**Injection volume:** 60**Detector:** E, BAS LC-4B (Bioanalytical Systems), glassy carbon electrode +0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM**Retention time:** 8**Internal standard:** 9a-N-propargyl analog of azithromycin (10)**Limit of detection:** 100 ng/g (tissue)**Limit of quantitation:** 10 ng/mL (serum)

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDS

serum; human; mouse; rat; dog; rabbit; brain; muscle; liver; kidney

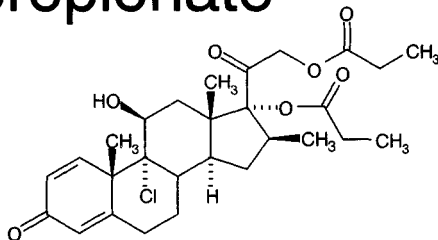
REFERENCEShepard, R.M.; Duthu, G.S.; Ferraina, R.A.; Mullins, M.A. High-performance liquid chromatographic assay with electrochemical detection for azithromycin in serum and tissues. *J.Chromatogr.*, **1991**, *565*, 321-337

Beclomethasone Dipropionate

Molecular formula: C₂₈H₃₇ClO₇

Molecular weight: 521.1

CAS Registry No.: 5334-09-8 (beclomethasone dipropionate), 4419-39-0 (beclomethasone)



SAMPLE

Matrix: blood

Sample preparation: 50 μ L Plasma + 100 μ L 20 μ g/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 μ L mobile phase, inject a 50 μ L 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

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 μ L 20 μ g/mL IS, extract with 8 mL dichloromethane. Evaporate the organic layer to dryness under vacuum, reconstitute in 120 μ L MeOH:5% acetic acid 50:50, inject an 80 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax ODS C18

Mobile phase: MeCN:MeOH:water 44:11:45

Flow rate: 1

Injection volume: 80

Detector: UV 242; Radioactivity

CHROMATOGRAM

Internal standard: hydrocortisone 21-S-propionate (JO 498)

OTHER SUBSTANCES

Extracted: metabolites, budesonide

KEY WORDS

rat; lung; radiolabeled; pharmacokinetics; plasma

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: 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: 8.78

OTHER SUBSTANCES

Simultaneous: bamipine lactate, betamethasone-17-valerate, dexamethasone, 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: 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; UV 238

CHROMATOGRAM

Retention time: 21.3
Internal standard: 17-hydroxyprogesterone (6.0), 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: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax SAX
Mobile phase: MeOH:buffer 50:50 (Buffer was 180 mM Na₂HPO₄ adjusted to pH 3.00 ± 0.05 with 180 mM orthophosphoric acid. Pass mobile phase through a 250 × 4.6 25-40 μm silica (HPLC Technology) column to saturate it with silica.)
Flow rate: 1
Detector: UV 253

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Simultaneous: cromolyn, minocromil, nedocromil, quinoline yellow, saccharin, salicylic acid
Interfering: acetaminophen, albuterol, aspartame, aspirin, caffeine, isoproterenol, menthol, reproterol, riboflavin, sorbitan trioleate, terbutaline, theophylline

REFERENCE

Baker, P.R.; Gardner, J.J.; Wilkinson, D. Automated high-performance liquid chromatographic method for the determination of nedocromil sodium in human urine using bimodal column switching. *J.Chromatogr.B*, **1995**, *668*, 59-65

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: present 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

lung

REFERENCE

Ronca-Testoni, S. Hydrolysis of cyclomethasone by the human lung. *Int.J.Clin.Pharmacol.Res.*, **1983**, *3*, 17–20

ANNOTATED BIBLIOGRAPHY

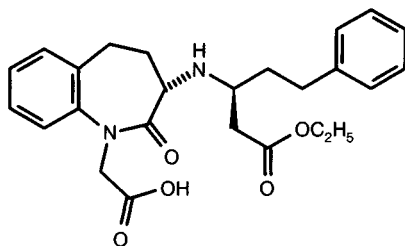
- Valvo, L.; Paris, A.; Savella, A.L.; Gallinella, B.; Ciranni Signoretti, E. General high-performance liquid chromatographic procedures for the rapid screening of natural and synthetic corticosteroids. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 805–810 [gradient; reverse phase; normal phase; also betamethasone, betamethasone 21-acetate, betamethasone 17,21-dipropionate, betamethasone 21-disodium phosphate, betamethasone 17-valerate, cortisone, cortisone 21-acetate, 11-deoxycorticosterone 21-acetate, dexamethasone, dexamethasone 21-acetate, dexamethasone 21-disodium phosphate, fluocinolone, fluocinolone acetonide, 9 α -fluorohydrocortisone, 9 α -fluorohydrocortisone 21-acetate, 9 α -fluoroprednisolone, 9 α -fluoroprednisolone 21-acetate, hydrocortisone, hydrocortisone 21-acetate, hydrocortisone 21-hemisuccinate, 6 α -methylprednisolone, 6 α -methylprednisolone 21-acetate, 6 α -methylprednisolone 21-sodium succinate, prednisolone, prednisolone 21-acetate, prednisolone 21-disodium phosphate, prednisolone 21-pivalate, prednisolone 21-sodium succinate, prednisone, triamcinolone, triamcinolone acetonide]
- Girault, J.; Istin, B.; Malgouyat, J.M.; Brisson, A.M.; Fourtillan, J.B. Simultaneous determination of beclomethasone, beclomethasone monopropionate and beclomethasone dipropionate in biological fluids using a particle beam interface for combining liquid chromatography with negative-ion chemical ionization mass spectrometry. *J.Chromatogr.*, **1991**, *564*, 43–53 [plasma; urine; LC-MS; LOQ 1 ng/mL]

Benazepril

Molecular formula: C₂₄H₂₈N₂O₅

Molecular weight: 424.5

CAS Registry No.: 86541-75-5 (benazepril),
86541-74-4 (benazepril hydrochloride)



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)) KH₂PO₄ 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 239

CHROMATOGRAM

Retention time: 5.01

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benperidol, benzocaine, benzoylegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, ciben-zoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam,

nitrendipine, nizatidine, nortriptyline, omeprazole, pipramol, oxazepam, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proganil, promethazine, propafenone, propranolol, protriptyline, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: acenocoumarol, benazepril, chlordiazepoxide, clorazepate, dipyridamole, metapramine, mexiletine, nomifensine, oxprenolol, pipamperone, pyrimethamine, ticlopidine, trazodone, vincristine, vindesine, warfarin

KEY WORDS

whole blood; plasma

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 C2 SPE cartridge with 2 mL MeOH and 2 mL water. Adjust pH of plasma to 1.0 with dilute phosphoric acid, add 1 mL to the SPE cartridge, wash with pH 1.0 dilute phosphoric acid, wash with water, wash with EtOH, wash with MeCN, air dry, elute with 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Newguard-Phenyl

Column: 150 \times 3.9 μ Bondapak phenyl

Mobile phase: MeOH:water 49:51 with 1 vial PIC-A reagent/L, adjusted to pH 7.0 with phosphoric acid

Flow rate: 1

Detector: UV 210

KEY WORDS

plasma; dog; SPE; pharmacokinetics

REFERENCE

Kim, J.S.; Oberle, R.L.; Krummel, D.A.; Dressman, J.B.; Fleisher, D. Absorption of ACE inhibitors from small intestine and colon. *J. Pharm. Sci.*, **1994**, *83*, 1350–1356

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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: 14

OTHER SUBSTANCES

Simultaneous: captopril, cilazapril, enalapril, quinapril, ramipril

REFERENCE

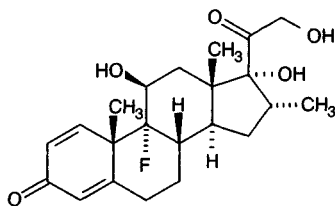
Barbato, F.; Morrica, P.; Quaglia, F. Analysis of ACE inhibitor drugs by high performance liquid chromatography. *Farmaco*, **1994**, *49*, 457-460

Betamethasone

Molecular formula: C₂₂H₂₉FO₅

Molecular weight: 392.5

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)



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 NaH₂PO₄ 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; UV 256; UV 343

CHROMATOGRAM

Retention time: 19.45

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: chloroquine, corticosterone, cortisone, dexamethasone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17β-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 method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, 1995, 666, 347-353

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 + 200 µL isopropanol:acetonitrile 1:1, mix, add to SPE cartridge, wash with 10 mL water, elute with 3 mL MeOH. Evap-

orate 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: 33

Internal standard: betamethasone

OTHER SUBSTANCES

Simultaneous: metabolites, cortisone, hydrocortisone, prednisolone, prednisone

KEY WORDS

serum; SPE; betamethasone is IS

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: 1 mL Plasma + 15 mL dichloromethane, shake horizontally for 15 min, centrifuge at 1500 g for 15 min. Remove the organic layer and wash it with 100 µL 100 mM NaOH then 1 mL water. Remove the aqueous phase and dry the organic phase over 1 g of anhydrous sodium sulfate. Evaporate the organic phase to dryness under a stream of nitrogen at not more than 37°, reconstitute in 200 µL mobile phase, inject a 175 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 30-38 µm HC Pellosil

Column: 250 × 4.6 5-6 µm Zorbax SIL

Mobile phase: Heptane:dichloromethane:glacial acetic acid:ethanol 350:600:10:35

Flow rate: 2

Injection volume: 175

Detector: UV 254

CHROMATOGRAM

Retention time: 12

Internal standard: betamethasone

OTHER SUBSTANCES

Extracted: hydrocortisone, prednisolone, prednisone

Noninterfering: cyclosporin, ethinyl estradiol, ketoconazole, levonorgestrel, rapamycin, tacrolimus, tenidap, tetrahydrocortisone

KEY WORDS

plasma; normal phase; betamethasone is IS

REFERENCE

Jusko, W.J.; Pyszczyński, N.A.; Bushway, M.S.; D'Ambrosio, R.; Mis, S.M. Fifteen years of operation of a high-performance liquid chromatographic assay for prednisolone, cortisol and prednisone in plasma. *J.Chromatogr.B*, **1994**, *658*, 47–54

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 μ L MeCN:water 30:70, inject whole sample.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:water 30:70

Flow rate: 1

Injection volume: 50

Detector: enzyme immunoassay of fractions

CHROMATOGRAM

Retention time: 16

Limit of detection: 0.3 pg

OTHER SUBSTANCES

Extracted: dexamethasone, 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: 1 mL Plasma + 5 mL water + 1 mL 2 μ g/mL equilenin in MeOH + 50 μ L 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 μ L mobile phase, inject 25 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak 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, prednisolone, prednisone, triamcinolone

Interfering: dexamethasone

KEY WORDS

Anal. Abs. 1982, 43, 4D182; 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: 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: 5.17 (betamethasone-17-valerate)

OTHER SUBSTANCES

Simultaneous: bampine lactate, beclomethasone dipropionate, dexamethasone, 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 bampine in pharmaceutical formulations by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1995**, 18, 3507-3517

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 \times 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: 6

Internal standard: cortisone (3)

OTHER SUBSTANCES

Simultaneous: dexamethasone, hydrocortisone, 6 α -methylprednisolone, prednisolone, prednisone

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 betamethasone and dexamethasone. *J.Chromatogr.A*, **1994**, 676, 455-460

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 \times 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: 7

Internal standard: phenacetin (10)

Limit of detection: 4.2 pmol

OTHER SUBSTANCES

Simultaneous: dexamethasone

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:** 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:** 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:** dexamethasone, flumethasone 21-acetate

KEY WORDSSPE; for betamethasone 17-valerate

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:** 500 × 1 C18 (Alltech)**Mobile phase:** MeOH:water 65:35**Flow rate:** 0.04**Injection volume:** 0.5**Detector:** UV 254; MS, Hewlett Packard 5985, home-made interface (details in paper)

CHROMATOGRAM**Retention time:** 21

OTHER SUBSTANCES

Simultaneous: metabolites, hydrocortisone

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, tissue

Sample preparation: Buffer solutions. Condition a 3 mL Baker C18 SPE cartridge with 400 μ L MeOH. 100-500 μ L 0.1 M pH 4.5 acetate buffer containing steroids + 100-1000 ng prednisone in MeOH, add to SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of air, reconstitute in 40-200 μ L dichloromethane:MeOH 98:2, inject a 40 μ L aliquot. Skin tissue. Crush tissue with 3 mL MeOH:100 mM pH 4.5 acetate buffer 20:80 using a Polytron tissue homogenizer, wash homogenizer twice with the same solution, combine all solutions, add 400-4000 ng prednisone in MeOH, add 10 mL dichloromethane, vortex for 1 min, centrifuge at 3000 rpm for 5 min. Filter the organic phase through a column of Celite 545, evaporate to dryness under a stream of air, reconstitute in 40-200 μ L dichloromethane:MeOH 98:2, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 μ m LiChrosorb Si-60

Mobile phase: n-Hexane:dichloromethane:MeOH:water 63.9:30:6:0.1

Flow rate: 1.2

Injection volume: 40-200

Detector: UV 240

CHROMATOGRAM

Retention time: 6.6

Internal standard: prednisone (5.3)

Limit of detection: 4 ng

KEY WORDS

SPE; normal phase

REFERENCE

Kubota, K.; Maibach, H.I. In vitro percutaneous permeation of betamethasone and betamethasone 17-valerate. *J.Pharm.Sci.*, **1993**, *82*, 1039-1045

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 μ L 5 μ g/mL IS in MeOH, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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

Extracted: corticosterone, cortisone, fluorocortisone, fluorocortisone acetate, hydrocortisone, hydroxyprogesterone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide
Interfering: dexamethasone

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: Dilute, if necessary, 100 μL -1 mL urine to 1 mL with water, add to a Chem Elut high surface-area diatomaceous earth extraction column, after 5 min elute with two 6 mL portions of ethyl acetate, combine the eluates and wash them twice with 1 mL 200 mM NaOH. Dry the organic layer over 1 g anhydrous sodium sulfate, evaporate to dryness at 30° under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 70 \times 6 37-53 μm HC-Pellocil
Column: 250 \times 4.6 5-6 μm Zorbax SIL
Mobile phase: Dichloromethane:glacial acetic acid:MeOH 91.3:7.5:1.2
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 8.5
Internal standard: betamethasone

OTHER SUBSTANCES

Extracted: metabolites, hydrocortisone, 6 β -hydroxycortisol, 20 β -hydroxyprednisone, 6 β -hydroxyprednisolone, 20 α -hydroxyprednisolone, 20 β -hydroxyprednisolone, prednisolone, prednisone

KEY WORDS

betamethasone is IS; normal phase

REFERENCE

Garg, V.; Jusko, W.J. Simultaneous analysis of prednisone, prednisolone and their major hydroxylated metabolites in urine by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, 567, 39-47

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 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 μ L MeOH, filter (0.45 μ m), inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 60 \times 4.6 3 μ m Hypersil ODS

Mobile phase: Gradient. MeOH:150 mM ammonium acetate from 40:60 to 50:50 over 6 min, maintain at 50:50 for 1 min, to 60:40 over 3 min, maintain at 60:40 for 5 min

Flow rate: 0.8

Injection volume: 15

Detector: MS, Hewlett-Packard HP 5988A, vaporizer probe 92° decreased to 89° over 6 min, decreased to 86° over 3 min, maintain at 86° for 5 min, ion source 276°, emission current 150 μ A, electron energy 955 eV, positive ion mode, filament on

CHROMATOGRAM

Retention time: 7

Internal standard: betamethasone

OTHER SUBSTANCES

Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 11 α -hydroxyprogesterone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide

KEY WORDS

betamethasone is IS

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

ANNOTATED BIBLIOGRAPHY

Jönsson, G.; Åström, A.; Andersson, P. Budesonide is metabolized by cytochrome P450 3A (CYP3A) enzymes in human liver. *Drug Metab.Dispos.*, **1995**, *23*, 137–142 [human; liver; microsomal incubations; extracted budesonide; betamethasone is IS; SPE; gradient]

Garg, V.; Jusko, W.J. Effects of indomethacin on the pharmacokinetics and pharmacodynamics of prednisolone in rats. *J.Pharm.Sci.*, **1994**, *83*, 747–750 [rat; plasma; extracted corticosterone, prednisolone, prednisone; betamethasone is IS]

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 [interfering triamcinolone acetonide; simultaneous corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fludrocortisone, fludrocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, 11 α -hydroxyprogesterone, methylprednisolone, prednisolone, prednisone, triamcinolone]

Santos-Montes, A.; Gasco-López, A.I.; Izquierdo-Hornillos, R. Simultaneous determination of dexamethasone and betamethasone in pharmaceuticals by reversed-phase HPLC. *Chromatographia*, **1994**, *39*, 539–542 [simultaneous dexamethasone; methylprednisolone (IS); tablets; column temp 30; LOD 6 ng/mL; non-interfering corticosterone, cortisone, deflazacort, fludrocortisone, fludrocortisone acetate, hydrocortisone, hydroxyprogesterone, methylprednisolone, prednisolone, prednisone, triamcinolone; interfering triamcinolone acetonide]

Valvo, L.; Paris, A.; Savella, A.L.; Gallinella, B.; Ciranni Signoretti, E. General high-performance liquid chromatographic procedures for the rapid screening of natural and synthetic corticosteroids. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 805–810 [for betamethasone, betamethasone 21-acetate, betamethasone 17,21-dipropionate, betamethasone 21-disodium phosphate, betamethasone 17-valerate; gra-

dient; reverse phase; normal phase; also beclomethasone, beclomethasone 17,21-dipropionate, cortisone, cortisone 21-acetate, 11-deoxycorticosterone 21-acetate, dexamethasone, dexamethasone 21-acetate, dexamethasone 21-disodium phosphate, fluocinolone, fluocinolone acetonide, 9 α -fluorohydrocortisone 21-acetate, 9 α -fluorohydrocortisone, 9 α -fluoroprednisolone, 9 α -fluoroprednisolone 21-acetate, hydrocortisone, hydrocortisone 21-acetate, hydrocortisone 21-hemisuccinate, 6 α -methylprednisolone, 6 α -methylprednisolone 21-acetate, 6 α -methylprednisolone 21-sodium succinate, prednisolone, prednisolone 21-acetate, prednisolone 21-disodium phosphate, prednisolone 21-pivalate, prednisolone 21-sodium succinate, prednisone, triamcinolone, triamcinolone acetonide]

Santos-Montes, A.; Gasco-Lopez, A.I.; Izquierdo-Hornillos, R. Optimization of the high-performance liquid chromatographic separation of a mixture of natural and synthetic corticosteroids. *J.Chromatogr.*, **1993**, *620*, 15–23 [simultaneous corticosterone, cortisone, deoxycorticosterone, dexamethasone, fluorocortisone, hydrocortisone, hydroxyprogesterone, methylprednisolone, prednisolone, prednisone, triamcinolone]

Smith, E.W.; Haigh, J.M. In vitro diffusion cell design and validation. I. A stability-indicating high-performance liquid chromatographic assay for betamethasone 17-valerate in purified isopropyl myristate receptor phase. *Pharm.Res.*, **1989**, *6*, 431–435

Maron, N.; Cristi, E.A.; Ramos, A.A. Determination of betamethasone 17-benzoate in lipophilic vehicles by reversed-phase high-performance liquid chromatography. *J.Pharm.Sci.*, **1988**, *77*, 638–639

Skrabalak, D.S.; Cuddy, K.K.; Henion, J.D. Quantitative determination of betamethasone and its major metabolite in equine urine by micro-liquid chromatography-mass spectrometry. *J.Chromatogr.*, **1985**, *341*, 261–269

Tokunaga, H.; Kimura, T.; Kawamura, J. Determination of glucocorticoids by liquid chromatography. III. Application to ointments and a cream containing cortisone acetate, dexamethasone acetate, fluorometholone, and betamethasone valerate. *Chem.Pharm.Bull.*, **1984**, *32*, 4012–4016

Cairns, T.; Siegmund, E.G.; Stamp, J.J.; Skelly, J.P. Liquid chromatography mass spectrometry of dexamethasone and betamethasone. *Biomed.Mass.Spectrom.*, **1983**, *10*, 203–208

Okumura, T. Application of thin-layer chromatography to high-performance liquid chromatographic separation of steroidal hormones and cephalosporin antibiotics. *J.Liq.Chromatogr.*, **1981**, *4*, 1035–1064 [normal phase; also cephalixin, cephaloglycine, cephaloridine, cephalothin, cortisone, dexamethasone, hydrocortisone]

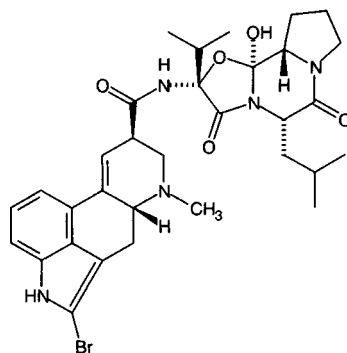
Petersen, M.C.; Nation, R.L.; Ashley, J.J. Simultaneous determination of betamethasone, betamethasone acetate and hydrocortisone in biological fluids using high-performance liquid chromatography. *J.Chromatogr.*, **1980**, *183*, 131–139

Bromocriptine

Molecular formula: C₃₂H₄₀BrN₅O₅

Molecular weight: 654.6

CAS Registry No.: 25614-03-3, 22260-51-1 (mesylate)



SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 1 mL Plasma + 10 μ L 50 μ g/mL ergocriptine in MeCN: MeOH:water 4:1:5 + 1 mL 2.5 M potassium carbonate, vortex, slowly add 4 mL MeCN with vortexing for 30 s, centrifuge at 2200 g at -10° for 15 min, maintain at -10° for 30 min. Remove the organic layer and evaporate it to dryness, dissolve residue in 100 μ L MeCN:MeOH:water 4:1:5, filter (0.45 μ m), inject a 50 μ L aliquot. Tissue. Sonicate 300 mg rat brain tissue in 1 mL water (Heat Systems-Ultrasonics), add 10 μ L 10 μ g/mL ergocriptine in MeCN:MeOH:water 4:1:5, add 1 mL 2.5 M aqueous potassium carbonate, extract with 4 mL MeCN, centrifuge at 2200 g at -10° for 15 min, maintain at -10° for 30 min. Remove the organic layer and evaporate it to dryness, dissolve residue in 100 μ L MeCN:MeOH:water 4:1:5, filter (0.45 μ m), inject a 50 μ L aliquot. (All glassware should be silanized.)

HPLC VARIABLES

Column: 70 \times 4.6 3 μ m Ultrasphere XL C8

Mobile phase: MeCN:isopropanol:25.3 mM ammonium carbonate 40:6:54 (After each run elute column with MeCN:water 80:20 for 15 min.)

Flow rate: 1.2

Injection volume: 50

Detector: UV 310

CHROMATOGRAM

Retention time: 10.5

Internal standard: ergocriptine (6)

Limit of detection: 19.5 ng/mL (plasma); 65 ng/g (tissue)

KEY WORDS

plasma; rat; brain

REFERENCE

Phelan, D.G.; Greig, N.H.; Rapoport, S.I.; Soncrant, T.T. High-performance liquid chromatographic assay of bromocriptine in rat plasma and brain. *J.Chromatogr.*, **1990**, 533, 264–270

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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), 6.21 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, 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, methyl-dopa, methylphenidate, metoclopramide, metolazine, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethyl-pyrazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 × 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: 5.5

OTHER SUBSTANCES

Simultaneous: benztropine mesylate, biperiden, desipramine, hyoscyamine, orphenadrine

Noninterfering: amantadine, carbidopa, levodopa

REFERENCE

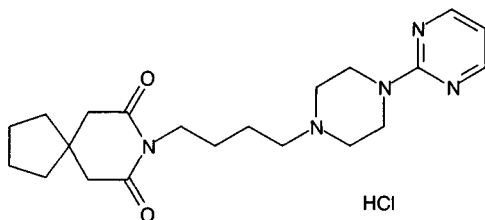
Selinger, K.; Lebel, G.; Hill, H.M.; Discenza, C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma. *J.Chromatogr.*, **1989**, *491*, 248–252

Buspirone

Molecular formula: C₂₁H₃₁N₅O₂

Molecular weight: 385.5

CAS Registry No.: 36505-84-7 (buspirone),
33386-08-2 (buspirone hydrochloride)



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 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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 236

CHROMATOGRAM

Retention time: 5.39

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Also analyzed: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprolol, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, lorazepam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephensesin, mepenthermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, ni-

flumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfipyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, videsine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

KEY WORDS

whole blood; plasma

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 Baker carboxylic acid SPE cartridge with 5 mL 2 M HCl and 10 mL water. Mix 500 μ L plasma + 5 mL water and add to column. Wash with 5 mL water and elute with 2 mL 1 M formic acid. Evaporate eluate to dryness under vacuum and dissolve residue in 200 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 5 μ m Cyanonitrile

Column: 33 \times 4.6 Supelcosil LC-CN

Mobile phase: MeCN:20 mM pH 7 potassium phosphate buffer 43:57 adjusted to pH 7.34 with 0.5 M KOH

Flow rate: 0.4

Injection volume: 20

Detector: E, ESA Coulochem model 5100 A, two porous graphite working electrodes and associated palladium reference electrodes, +0.55 V for first electrode, +0.70 V for second (monitoring) electrode

CHROMATOGRAM

Retention time: 10

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, 1-(2-pyrimidinyl)piperazine

KEY WORDS

plasma; mouse; SPE

REFERENCE

Betto, P.; Meneguz, A.; Ricciarello, G.; Pichini, S. Simultaneous high-performance liquid chromatographic analysis of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in plasma using electrochemical detection. *J. Chromatogr.*, **1992**, *575*, 117–121

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL C18 Supelco SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 50 mM KH_2PO_4 adjusted to pH 7.2 with 2 M NaOH, add 2 mL serum and flush through 2-5 mL air. Wash with two 1 mL portions of 50 mM KH_2PO_4 adjusted to pH 7.2 with 2 M NaOH, wash with two 1 mL and one 0.5 mL portions of MeOH:water 1:1 and flush through with 2-5 mL of air. Elute with 1 mL MeCN:triethylamine 99:1. Evaporate solvent at 37° under a stream of air and dissolve residue in 100 μL of MeCN:5 mM KH_2PO_4 + 0.1% triethylamine adjusted to pH 2.5 with orthophosphoric acid 45:55, centrifuge at 5000 g for 10 min, inject a 70 μL aliquot onto column A with mobile phase A and elute to waste, switch a 0.7 min fraction onto column B (retention time is ca. 3.6 min) and chromatograph on column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 150 \times 4.6 5 μm Spherisorb ODS2; B 150 \times 4.6 5 μm Spherisorb ODS2
Mobile phase: A MeCN:5 mM KH_2PO_4 + 0.1% triethylamine adjusted to pH 2.5 with orthophosphoric acid 45:55; B MeCN:5 mM KH_2PO_4 + 0.2% triethylamine adjusted to pH 2.5 with orthophosphoric acid 55:45, containing 5 mM sodium lauryl sulfate
Flow rate: 1.2
Injection volume: 70
Detector: UV 235

CHROMATOGRAM

Retention time: 7 (on column B)
Limit of detection: 0.2 ng/mL

KEY WORDS

serum; column-switching; heart-cut; SPE; pharmacokinetics

REFERENCE

Kristjánsson, F. Sensitive determination of buspirone in serum by solid-phase extraction and two-dimensional high-performance liquid chromatography. *J.Chromatogr.*, **1991**, 566, 250-256

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 50 μL 400 ng/mL gepirone in EtOH + 0.5 mL 1 M NaOH + 4 mL ethyl acetate, shake slowly mechanically for 10 min, centrifuge at 400 g for 10 min. Freeze lower plasma layer in dry ice/acetone and decant ethyl acetate into a centrifuge tube containing 2 mL 50 mM HCl, shake 10 min, centrifuge 400 g 10 min, remove, discard organic phase. Add 500 μL 4 M ammonia solution and 400 μL butyl acetate to the aqueous phase, mix for 15-20 sec, centrifuge at 400 g for 10 min. Remove organic layer and evaporate it to dryness at 30° under vacuum. Reconstitute in 90 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm Cyanonitrile (Anachem)
Column: 10 \times 4.6 5 μm cyanonitrile (Brownlee)
Mobile phase: MeCN:40 mM potassium phosphate buffer adjusted to pH 6.6 with 2 M KOH 34:66 (Columns were initially conditioned with 50 mL water; then 5 mM pH 4.8 sodium acetate buffer; then MeCN:5 mM pH 4.8 sodium acetate buffer 40:60; then 5 mM pH 4.8 potassium acetate; then mobile phase.)
Flow rate: 1.5
Injection volume: 50
Detector: E, ESA Coulochem model 5100 A, Model 5020 guard cell, guard cell 0.3 V, detector 0.55 V for first electrode and 0.70 V for second electrode

CHROMATOGRAM

Retention time: 7.3

Internal standard: gepirone (5.4)

Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Simultaneous: 1-(2-pyrimidinyl)piperazine, metabolites, amitriptyline, chlorpromazine, clomipramine, fluphenazine, imipramine

Noninterfering: caffeine, diazepam, desipramine, mianserin, zimeldine

Interfering: haloperidol

KEY WORDS

plasma

REFERENCE

Franklin, M. Determination of plasma buspirone by high-performance liquid chromatography with coulometric detection. *J.Chromatogr.*, **1990**, 526, 590–596

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μ L 50 μ g/mL 1-phenylpiperazine + 1 mL pH 10 borate buffer + 5 mL chloroform:MeCN 8:2, agitate, centrifuge, repeat extraction. Evaporate organic layers under reduced pressure below 40°, dissolve residue in 100 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 23 \times 3.6 37-50 μ m CN/Corasil

Column: 250 \times 4.6 5 μ m Spherisorb CN

Mobile phase: MeOH:5 mM KH₂PO₄ pH 7.4 35:65

Flow rate: 1.7

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 10

Internal standard: 1-phenylpiperazine (13)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: 1-(2-pyrimidinyl)piperazine, metabolites

KEY WORDS

plasma; rat

REFERENCE

Diaz-Marot, A.; Puigdellivol, E.; Salvatella, C.; Comellas, L.; Gassiot, M. Determination of buspirone and 1-(2-pyrimidinyl)piperazine in plasma samples by high-performance liquid chromatography. *J.Chromatogr.*, **1989**, 490, 470–473

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4.5 μ m 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: 9.07 (A), 4.98 (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, 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, methyl-dopa, 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, pimozone, 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, sulfinyprazone, 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

some 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 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, 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, fenopropfen, 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, isocarboxystiril, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, 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, pyrillamine, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, 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

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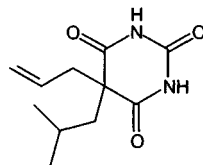
- Gil, M.S.; Ochoa, C.; Vega, S. High performance liquid chromatography of new potential anxiolytic drugs and related benzodiazepines: A comparative study of hydrophobicity. *J.Liq.Chromatogr.*, **1991**, *14*, 2141–2156 [also chlordiazepoxide, diazepam]
- Sarati, S.; Guiso, G.; Spinelli, R.; Caccia, S. Determination of piribedil and its basic metabolites in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, *563*, 323–332 [plasma; rat; extracted piribedil; buspirone is IS; gradient]

Butalbital

Molecular formula: C₁₁H₁₆N₂O₃

Molecular weight: 224.3

CAS Registry No.: 77-26-9



SAMPLE

Matrix: blood

Sample preparation: 1 mL Blood + 1 mL water + 50 μ L 76 mg/L allobarbital in EtOH: water 10:90 + 5 mL ethyl acetate, shake by hand, add 2 mL of 0.1 M HCl. Mix by inversion with a mechanical shaker for 5 min. Centrifuge at 2700 rpm for 5-10 min. Remove ethyl acetate and evaporate to dryness under a stream of nitrogen at room temperature. Take up in 200 μ L MeOH, filter (0.45 μ m), inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: Guard-Pak precolumn insert

Column: 200 \times 4.6 5 μ m Hypersil octadecylsilane

Mobile phase: Gradient. MeCN: 1 mM pH 3.2 phosphate buffer from 20:80 to 40:60 over 10 min, stay at 40:60 for 6 min, to 20:80 over 4 min.

Column temperature: 60

Flow rate: 3

Injection volume: 20

Detector: UV 202

CHROMATOGRAM

Retention time: 5.0

Internal standard: allobarbital (2.9)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: salicylic acid

Simultaneous: aspirin, caffeine

KEY WORDS

pharmacokinetics

REFERENCE

Drost, M.L.; Walter, L. Blood and plasma concentrations of butalbital following single oral doses in man. *J. Anal. Toxicol.*, 1988, 12, 322-324

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 200 μ L 50 μ g/mL hexobarbital in MeCN + 25 μ L glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: Gradient. MeCN: 7.5 g/L NaH₂PO₄ 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: 17.1

Internal standard: hexobarbital (20.6)

Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, 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: 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: 5.77 (A), 5.12 (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, 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, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, fu-roseamide, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, mida-

zolam, 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, pimizide, 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, sulfapyrazone, 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

some 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: 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' 1.15

OTHER SUBSTANCES

Simultaneous: amobarbital, aprobarbital, butobarbital, pentobarbital, phenobarbital, secobarbital, thiopental

REFERENCE

Forgács, E.; Cserhádi, 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

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; UV 270

CHROMATOGRAM**Retention time:** 11.25

OTHER SUBSTANCES**Extracted:** amobarbital, butabarbital, butethal, pentobarbital, talbutal

KEY WORDSmass spectra given

REFERENCERyan, 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:** 300 × 3.9 μBondapak C18**Mobile phase:** MeCN:10 mM KH₂PO₄ + 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:** 7.9**Internal standard:** methyl paraben (7.0)**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES**Simultaneous:** allobarbital, aprobarbital, barbital, mephobarbital, pentobarbital, phenobarbital, secobarbital, talbutal, vinbarbital

KEY WORDSstability-indicating

REFERENCEIbrahim, 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

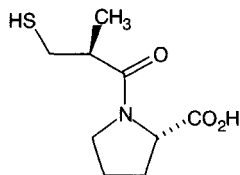
ANNOTATED BIBLIOGRAPHYRyan, T.W. Resolution of the non-specific spectra of barbiturates by UV-photodiode array detection. *J.Liq.Chromatogr.*, **1993**, *16*, 315–329 [allobarbital, amobarbital, barbital, mephobarbital, pentobarbital, phenobarbital, secobarbital, talbutal, vinbarbital]

Captopril

Molecular formula: C₉H₁₅NO₃S

Molecular weight: 217.3

CAS Registry No.: 62571-86-2



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 30 μ L 1 mg/mL p-bromophenacyl bromide in MeCN + 50 μ L 100 mM NaOH, shake for 15 min, add 75 μ L 1 M HCl, add 500 ng nitrazepam for each mL, add 150 μ L 200 mM pH 4.0 acetate buffer. Extract with 4 mL benzene (Caution! Benzene is a carcinogen!), centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Kontron Analytical S5 ODS2

Mobile phase: MeCN:1% acetic acid 60:40

Flow rate: 1.3

Injection volume: 20

Detector: UV 260

CHROMATOGRAM

Retention time: 4

Internal standard: nitrazepam (4.5)

Limit of detection: 15 ng/mL

Limit of quantitation: 30 ng/mL

KEY WORDS

plasma; derivatization; pharmacokinetics

REFERENCE

Jankowski, A.; Skorek, A.; Krzysko, K.; Zarzycki, P.K.; Ochocka, R.J.; Lamparczyk, H. Captopril: determination in blood and pharmacokinetics after single oral dose. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 655-660

SAMPLE

Matrix: blood

Sample preparation: 1 mL Blood + 50 μ L solution containing 100 mM EDTA and 100 mM ascorbic acid, centrifuge at 13000 g for 2 min. 500 μ L supernatant + 2 mL 100 mM pH 7 phosphate buffer + 200 ng IS + 200 μ L 1.5 mg/mL N-(3-pyrenyl)maleimide in MeCN, shake for 15 min, acidify with 100 μ L 11 M HCl, add 6 mL ethyl acetate, vortex for 20 min, centrifuge at 2500 g for 5 min. Remove the organic layer, dry it under nitrogen, dissolve in 50 or 200 μ L MeCN, inject a 5-15 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m Partisil ODS-3 C18

Mobile phase: MeCN:1% acetic acid 37:63

Flow rate: 1.5

Injection volume: 5-15

Detector: F ex 340 em 389

CHROMATOGRAM

Retention time: 8

Internal standard: (4R)-2-(2-Hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA 446) (14)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: chloral hydrate, chlorpromazine, furosemide, digoxin, promethazine

KEY WORDS

with modifications can be used to determine captopril disulfide; derivatization; pharmacokinetics

REFERENCE

Pereira, C.M.; Tam, Y.K.; Collins-Nakai, R.L.; Ng, P. Simplified determination of captopril in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *425*, 208–213

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Whole blood + 20 μ L 1% N-(4-dimethylaminophenyl) maleimide in acetone + 300 μ L 33.3 mM pH 6.85 phosphate buffer, add 100 ng IS, vortex, let stand at 0° for 30 min, freeze in dry ice/acetone, thaw, wash twice with 2 mL portions of ether. Add 500 μ g glutathione to the aqueous layer, let stand at 0° for 20 min, add 3 mL acetone, centrifuge at 1580 g for 5 min. Remove the supernatant and wash the precipitate with 3 mL acetone. Combine the supernatant and the wash and evaporate them to about 1 mL under reduced pressure at room temperature, dilute the residue with 6 mL water, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL water, elute with 8 mL MeCN. Evaporate the eluate to dryness under reduced pressure below 40°, reconstitute the residue in 200 μ L MeOH, inject an aliquot. (Prepare N-(4-dimethylaminophenyl)maleimide as follows. Mix equimolar amounts of maleic anhydride and N, N-dimethyl-1,4-phenylenediamine in ether or THF with cooling in ice and stirring, allow to stand overnight, remove the maleamic acid by filtration, wash with THF. Heat 1 mmole of the maleamic acid with 3 mmole acetic anhydride and 0.3 mmole sodium acetate at 100° for 5-10 min (until the solution goes clear), cool, add ice water, neutralize with sodium bicarbonate, extract with ethyl acetate. Wash the organic layer with saturated sodium chloride and dry over anhydrous sodium sulfate (Chem. Pharm. Bull. 1976, 24, 3045; 1977, 25, 2739). Evaporate to dryness and recrystallize from acetone to give N-(4-dimethylaminophenyl)maleimide as reddish-brown crystals (mp 153-154° (J. Org. Chem. 1963, 28, 2018)).)

HPLC VARIABLES

Column: 300 \times 3.9 8-10 μ m μ Bondapak C18

Mobile phase: MeCN:0.8% pH 3.0 (NH₄)H₂PO₄ 1:2

Flow rate: 1

Detector: E, Yanagimoto VMD 101, +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 10

Internal standard: (4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (Sankyo SA 446) (19)

Limit of detection: 10 ng/mL

KEY WORDS

derivatization; whole blood; SPE; pharmacokinetics

REFERENCE

Shimada, K.; Tanaka, M.; Nambara, T.; Imai, Y.; Abe, K.; Yoshinaga, K. Determination of captopril in human blood by high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1982**, *227*, 445–451

SAMPLE**Matrix:** blood**Sample preparation:** 3 mL Blood + 1.5 mL 0.5% p-bromophenacyl bromide in acetone, vortex for 30 s, let stand for 5 min, acidify with 300 mM HCl, store below -15°, extract with 16 mL benzene (Caution! Benzene is a carcinogen!), extract with 8 mL benzene. Combine the organic layers and evaporate them to dryness under reduced pressure, reconstitute the residue in 4 mL 50 mM pH 7 phosphate buffer, add 20 mL hexane, sonicate, wash with 6 mL hexane, discard the hexane layer, acidify the aqueous layer with 100 μ L 2 M HCl, extract with 6 mL benzene, extract with 2 mL benzene. Combine the organic layers and add 500 ng IS, evaporate to dryness under reduced pressure, reconstitute the residue in 200 μ L MeCN, inject a 5-25 μ L aliquot.

HPLC VARIABLES**Column:** μ Bondapak C18**Mobile phase:** MeCN:water:acetic acid 48:51.5:0.5**Injection volume:** 5-25**Detector:** UV 254

CHROMATOGRAM**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.)**Limit of quantitation:** 5 ng/mL

KEY WORDS

derivatization; whole blood

REFERENCEKawahara, Y.; Hisaoka, M.; Yamazaki, Y.; Inage, A.; Morioka, T. Determination of captopril in blood and urine by high-performance liquid chromatography. *Chem.Pharm.Bull.*, **1981**, *29*, 150-157

SAMPLE**Matrix:** blood, CSF**Sample preparation:** 1 mL blood + 50 μ L of a solution containing 100 mM EDTA and 100 mM ascorbic acid, centrifuge at 13000 g for 2 min. Proceed immediately. 500 μ L Plasma or CSF + 50 μ L 1 mg/mL p-bromophenacyl bromide in acetonitrile, vortex for 15 s, leave at room temperature for 30 min, add 100 μ L 1 M HCl, add 50 μ L 0.02% phenylacetic acid in methanol, add 5 mL ethyl acetate:benzene 50:50 (Caution! Benzene is a carcinogen!), vortex for 3 min, shake gently for 15 min. Saturate the aqueous phase with NaCl, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute in 250 μ L MeCN, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 4.5 μ m Lichrosorb RP-18**Mobile phase:** MeCN:water:acetic acid 220:180:2.5**Flow rate:** 1; increase to 3 after captopril elutes**Injection volume:** 20**Detector:** UV 260

CHROMATOGRAM**Retention time:** 4.7**Internal standard:** phenylacetic acid (3.2)**Limit of detection:** 5 ng/mL

KEY WORDS

plasma; with modification can be used to determine captopril disulfides; derivatization

REFERENCE

Colin, P.; Scherer, E. Simple high-performance liquid chromatography determination of captopril in human plasma and cerebrospinal fluid. *J.Liq.Chromatogr.*, **1989**, *12*, 629–643

SAMPLE

Matrix: blood, urine

Sample preparation: Whole blood. 3 mL Whole blood + 100 μ L 100 mM EDTA + 100 μ L 200 mM ascorbic acid + 2 mL 1 M pH 8.2 Tris buffer + 200 μ L 3 μ g/mL IS + 100 μ L 20 μ g/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 μ L 3 M perchloric acid, centrifuge for 15 min, rinse the precipitate with 500 μ L 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 a conditioned Bakerbond C18 SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, elute with 200 μ L MeOH:acetic acid 80:20, elute with two 200 μ L portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μ L water, inject a 20 μ L aliquot. Urine. 500 μ L Urine + 100 μ L 200 mM EDTA + 100 μ L 200 mM ascorbic acid + 3 mL 1 M pH 8.2 Tris buffer + 200 μ L 3 μ g/mL IS + 100 μ L 20 μ g/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 μ L MeOH:acetic acid 80:20, elute with two 200 μ L portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μ L water, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 2.1 5 μ m Hypersil

Column: 150 \times 3.3 7 μ m 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 (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: Plasma. 1.5 mL Blood + 50 μ L 100 mM ascorbic acid and 100 mM disodium ethylenediaminetetraacetate, centrifuge. Remove 0.5 mL plasma and immediately add it to 50 μ L 1 mg/mL p-bromophenacyl bromide, vortex 30 s, let stand at room temperature for 20 min, add 300 μ L 6% perchloric acid, vortex for 30 s, centrifuge at 10000 g for 10 min, inject a 500 μ L aliquot onto column A with mobile phase A and elute to waste, after 3 min elute the contents of column A onto column B with mobile phase B, after 2 min remove column A, elute column B with mobile phase B, monitor the effluent from column B. Urine. 50 μ L Urine + 50 μ L 1 mg/mL p-bromophenacyl bromide + 600 μ L water, vortex 30 s, let stand at room temperature for 20 min, inject a 500 μ L aliquot onto column A with mobile phase A and elute to waste, after 3 min elute the contents of column A onto column B with mobile phase B, after 2 min remove column A, elute column B with mobile phase B, monitor the effluent from column B. (Column A should be washed with MeOH for 2 min then re-equilibrated with mobile phase A for 2 min.)

HPLC VARIABLES

Column: A 50 \times 5 37-50 μ m μ Bondapak C18; B 150 \times 5 5 μ m Tianjing silica gel YWG-C18

Mobile phase: A 0.2% Acetic acid in water; B MeCN:water:acetic acid 35:65:0.4

Flow rate: A 3; B 2

Injection volume: 500

Detector: UV 260

CHROMATOGRAM

Retention time: 9

Limit of detection: 10 ng/mL

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Gao, S.; Tian, W.; Wang, S. Simple high-performance liquid chromatographic method for the determination of captopril in biological fluids. *J.Chromatogr.*, **1992**, *582*, 258–262

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 2 mL 100 mM pH 6.0 phosphate buffer + 500 μ L 0.5% N-(4-benzoylphenyl)maleimide in acetone, vortex for 15 s, let stand at room temperature for 10 min, add 2 mL 500 mM pH 7.0 phosphate buffer, add 100 μ L 40 μ g/mL IS1 in acetone, wash twice with 4 mL portions of ether, acidify the aqueous phase with 500 μ L 6 M HCl, extract with 7 mL chloroform. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L MeOH, inject a 20 μ L aliquot. Urine. 200 μ L Urine + 200 μ L 0.5% N-(4-benzoylphenyl)maleimide in acetone + 200 μ L 100 mM pH 6.5 phosphate buffer, mix, let stand at room temperature for 15 min, add 2.5 mL 500 mM pH 7.0 phosphate buffer, wash with 4 mL diethyl ether, add 100 μ L 10 μ g/mL IS2 in acetone to the aqueous phase, acidify with 500 μ L 6 M HCl, extract with 6 mL chloroform. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μ L MeCN, inject a 20 μ L aliquot. (Prepare N-(4-benzoylphenyl)maleimide by adding 5.3 g maleic anhydride to 9.6 g 4-aminobenzophenone in dioxane (Caution! Dioxane is a carcinogen!), stir at room temperature (Japan Pat. 59,204,171 (19 Nov. 1984); Chem. Abstr. 1985, 102, 113288t).)

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:1% acetic acid 45:11:75 (plasma) or 42.5:8.2:47.3 (urine)

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM**Retention time:** 21 (plasma), 10 (urine)**Internal standard:** adduct of N-(4-benzoylphenyl)maleimide with (4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (IS1) 30 min), adduct of N-(4-benzoylphenyl)maleimide with thiosalicylic acid (IS2) (14 min) (Prepare adducts as follows. Add 150 mg N-(4-benzoylphenyl)maleimide in 2 mL acetone to 500 μ moles compound in 2 mL water, add 1 drop triethylamine, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure.)**Limit of detection:** 50 ng/mL

KEY WORDSderivatization; plasma; pharmacokinetics

REFERENCEHayashi, K.; Miyamoto, M.; Sekine, Y. Determination of captopril and its mixed disulfides in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, 338, 161-169

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute 5 fold with mobile phase. Mix the diluted formulation with an equal volume of 50 μ g/mL hydrochlorothiazide, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeOH:0.1% phosphoric acid 55:45**Flow rate:** 1**Injection volume:** 10**Detector:** UV 260

CHROMATOGRAM**Retention time:** 7.1**Internal standard:** hydrochlorothiazide (5.6)

OTHER SUBSTANCES**Simultaneous:** degradation products

KEY WORDSstability-indicating; syrup; water

REFERENCENahata, M.C.; Morosco, R.S.; Hipple, T.F. Stability of captopril in three liquid dosage forms. *Am.J.Hosp.Pharm.*, **1994**, 51, 95-96

SAMPLE**Matrix:** intestinal mucosal homogenate**Sample preparation:** 400 μ L Homogenate mixture + 400 μ L 1 M HCl, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μ m) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES**Guard column:** 20 mm long Supelguard LC-18S (Supelco)**Column:** 250 \times 4.6 Suplecasil 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:** perfusate**Sample preparation:** Centrifuge perfusate, add 100 μ L supernatant to 50 μ L 2 mM N-(1-pyrenyl)maleimide in acetone, add mixture to 2 mL pH 7 phosphate buffer, stir for 15 min at room temperature, inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Nucleosil 5C18**Mobile phase:** MeCN:0.1% phosphoric acid 47:53**Detector:** F ex 340 em 390

KEY WORDS

derivatization

REFERENCE

Kobayashi, D.; Matsuzawa, T.; Sugibayashi, K.; Morimoto, Y.; Kobayashi, M.; Kimura, M. Feasibility of use of several cardiovascular agents in transdermal therapeutic systems with *l*-menthol-ethanol system on hairless rat and human skin. *Biol.Pharm.Bull.*, **1993**, *16*, 254–258

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 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:** 4

OTHER SUBSTANCES**Simultaneous:** benzepiril, cilazapril, enalapril, quinapril, ramipril

REFERENCE

Barbato, F.; Morrica, P.; Quaglia, F. Analysis of ACE inhibitor drugs by high performance liquid chromatography. *Farmaco*, **1994**, *49*, 457–460

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 \times 3.9 μ Bondapak phenyl**Mobile phase:** MeOH:water:85% phosphoric acid 45:55:0.05**Column temperature:** 30-40**Detector:** UV 215-220

REFERENCE

Ranadive, S.A.; Chen, A.X.; Serajuddin, A.T. Relative lipophilicities and structural-pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors. *Pharm.Res.*, **1992**, *9*, 1480–1486

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 2 mL 500 mM pH 7.0 phosphate buffer + 0.5 mL 20 mg/mL p-bromophenacyl bromide in MeOH, shake vigorously. Remove a 1 mL aliquot and add it to 1.5 mL water and 6 mL hexane, mix, discard the hexane layer. Remove a 2 mL aliquot of the aqueous layer and add it to 100 μ L 2% tributylphosphine in MeOH, heat at 50° for 30 min, wash with 6 mL hexane, add 200 μ L 0.2% N-(4-dimethylamino-3,5-dinitrophenyl)maleimide in acetone to the aqueous layer, mix, let stand at room temperature for 5 min, wash with 6 mL hexane, discard the hexane layer, acidify the aqueous layer with about 200 μ L 2 M HCl, extract twice with 6 mL portions of benzene (Caution! Benzene is a carcinogen!). Combine the organic layers and add 10 μ g IS, evaporate to dryness under reduced pressure, reconstitute the residue in 200 μ L MeOH, inject a 5-20 μ L aliquot. (Free captopril is derivatized as its p-bromophenacyl bromide adduct then oxidized captopril is reduced and derivatized as its N-(4-dimethylamino-3,5-dinitrophenyl)maleimide adduct.)

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeCN: water: acetic acid 46.5:53:0.5

Injection volume: 5-20

Detector: UV 254

CHROMATOGRAM

Retention time: 7 (free captopril (as p-bromophenacyl bromide adduct)), 8 (oxidized captopril (as N-(4-dimethylamino-3,5-dinitrophenyl)maleimide adduct))

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.) (12.5)

Limit of quantitation: 100 ng/mL

KEY WORDS

derivatization

REFERENCE

Kawahara, Y.; Hisaoka, M.; Yamazaki, Y.; Inage, A.; Morioka, T. Determination of captopril in blood and urine by high-performance liquid chromatography. *Chem.Pharm.Bull.*, **1981**, *29*, 150–157

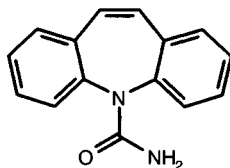
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Chan, D.S.; Sato, A.K.; Claybaugh, J.R. Degradation of captopril in solutions compounded from tablets and standard powder. *Am.J.Hosp.Pharm.*, **1994**, *51*, 1205–1207 [stability-indicating; tablets; powder]

Wakabayashi, H.; Yamato, S.; Nakajima, M.; Shimada, K. Application of an electrochemical detector with a graphite electrode to liquid chromatographic determination of penicillamine and captopril in biological samples. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1147–1152 [rat; serum; liver; kidney; SPE; electrochemical detection; LOD 20-300 pg; extracted penicillamine; homocysteine (IS)]

- Liu, C.; Chen, G. Quantitation determination of captopril intravenous injection by HPLC. *Zhongguo Yiyuan Yaoxue Zazhi*, **1992**, *12*, 170–171
- Tian, W.R.; Gao, S.; Wang, S.X. Determination of captopril in plasma and urine by high-performance liquid chromatography with column switching. *Yaoxue Xuebao*, **1992**, *27*, 613–617
- Jain, R.; Jain, C.L. Simultaneous quantification of captopril and hydrochlorothiazide using high performance liquid chromatography. *Indian Drugs*, **1991**, *28*, 380–382
- Tan, H.; Zhu, D.; Tang, S. HPLC determination in dissolution of captopril tablets. *Zhongguo Yaoxue Zazhi*, **1991**, *26*, 546–548
- 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
- Arzamastsev, A.P.; Volchenok, V.I.; Ordabaeva, S.K.; Ryzhenkova, A.P.; Nasyrov, S.N.; Shvarts, G.Y. HPLC determination of captopril in biological fluids. *Khim.-Farm.Zh.*, **1989**, *23*, 1404–1406
- Hu, M.; Amidon, G.L. Passive and carrier-mediated intestinal absorption components of captopril. *J.Pharm.Sci.*, **1988**, *77*, 1007–1011 [rat; perfusate; simultaneous captopril disulfide, cephadrine]
- Xu, Z. Determination of captopril by HPLC. *Yiyao Gongye*, **1985**, *16*, 536–538
- Kirschbaum, J.; Perlman, S. Analysis of captopril and hydrochlorothiazide combination tablet formulations by liquid chromatography. *J.Pharm.Sci.*, **1984**, *73*, 686–687
- Perrett, D.; Rudge, S.R.; Drury, P.L. Determination of captopril by an improved high-performance liquid chromatography-electrochemical assay. *Biochem.Soc.Trans.*, **1984**, *12*, 1059–1060
- Toyooka, T.; Imai, K.; Kawahara, Y. Determination of total captopril in dog plasma by HPLC after prelabeling with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F). *J.Pharm. Biomed.Anal.*, **1984**, *2*, 473–479 [derivatization]
- Perrett, D.; Drury, P.L. The determination of captopril in physiological fluids using high-performance liquid chromatography with electrochemical detection. *J.Liq.Chromatogr.*, **1982**, *5*, 97–110 [LOD 1 pmole; electrochemical detection; plasma; urine]

Carbamazepine



Molecular formula: C₁₅H₁₂N₂O

Molecular weight: 236.3

CAS Registry No.: 298-46-4

SAMPLE

Matrix: blood

Sample preparation: Add two volumes of MeCN to the mouse serum, mix, centrifuge at 1500 g for 5 min, inject a 5 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: Sentry (Waters)

Column: 150 \times 4.6 Nova-Pak C18

Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110

Column temperature: 40

Flow rate: 0.5

Injection volume: 5

Detector: UV 214

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Extracted: carbamazepine-10,11-epoxide, phenobarbital, phenylethyl malonamide, phenytoin, primidone

KEY WORDS

serum; mouse

REFERENCE

Capparella, M.; Foster, W., III; Larrousse, M.; Phillips, D.J.; Pomfret, A.; Tuvim, Y. Characteristics and applications of a new high-performance liquid chromatography guard column. *J.Chromatogr.A*, **1995**, *691*, 141-150

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma + 750 ng carbamazepine-10-hydroxide + 200 μ L 1.5 M NaOH + 2 mL ethyl acetate:chloroform 50:50, extract. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μ L mobile phase and 100 μ L hexane, inject a 20 μ L aliquot of the lower aqueous phase.

HPLC VARIABLES

Guard column: 50 \times 4.5 μ m Lichrospher 100 RP-18

Column: 125 \times 4.5 μ m Lichrocart C18

Mobile phase: MeCN:water 30:70

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Internal standard: carbamazepine-10-hydroxide

OTHER SUBSTANCES

Extracted: carbamazepine-10,11-epoxide, metabolites

KEY WORDS

plasma

REFERENCE

Lanchote, V.L.; Bonato, P.S.; Campos, G.M.; Rodrigues, I. Factors influencing plasma concentrations of carbamazepine and carbamazepine-10,11-epoxide in epileptic children and adults. *Ther. Drug Monit.*, **1995**, *17*, 47–52

SAMPLE

Matrix: blood

Sample preparation: Filter (0.22 μm), inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 4.5 μm LiChrospher 100 Diol

Mobile phase: MeCN:50 mM pH 6.9 phosphate buffer 12:88

Flow rate: 0.6

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 9.5

OTHER SUBSTANCES

Extracted: phenobarbital, phenytoin

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: Condition an Extrelut-1 glass SPE cartridge with 5 mL dichloromethane:isopropanol 90:10, dry under nitrogen. 1 mL Serum + 100 μL EtOH, vortex for 30 s, add to the SPE cartridge, let stand for 10 min, elute with 5 mL dichloromethane:isopropanol 90:10. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 10 μm Chiralcel OD + 250 \times 4.6 10 μm Chiralcel ODH

Mobile phase: n-Hexane:EtOH 70:30

Column temperature: 40 (2nd column)

Flow rate: 0.9

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 21.9

Internal standard: carbamazepine

OTHER SUBSTANCES**Extracted:** oxcarbazepine**Simultaneous:** phenobarbital**Noninterfering:** phenytoin, valproic acid**KEY WORDS**

serum; SPE; chiral (for oxcarbazepine metabolites); carbamazepine is IS

REFERENCE

Pichini, S.; Altieri, I.; Passa, A.R.; Zuccaro, P.; Pacifici, R. Stereoselective bioanalysis of oxcarbazepine and the enantiomers of its metabolites by high-performance liquid chromatography. *J.Liq. Chromatogr.*, **1995**, *18*, 1533–1541

SAMPLE**Matrix:** blood**Sample preparation:** 500 μL Serum + 6 mL MTBE, vortex for 30 s, shake for 5 min, centrifuge at 800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen in a warm water bath, reconstitute the residue in 40 μL MeOH: water 5:2, inject a 20 μL aliquot.**HPLC VARIABLES****Guard column:** 4 \times 4 5 μm LiChrospher 100 RP-18**Column:** 125 \times 4 4 μm Superspher 60 RP-select B (Merck)**Mobile phase:** MeCN:20 mM KH_2PO_4 20:80 containing 0.05% triethylamine, pH 6.30**Flow rate:** 1**Injection volume:** 20**Detector:** UV 212**CHROMATOGRAM****Retention time:** 27.43**Limit of detection:** 10 ng/mL**Limit of quantitation:** 39 ng/mL**OTHER SUBSTANCES****Extracted:** metabolites, oxcarbazepine**KEY WORDS**

serum

REFERENCE

Pienimäki, P.; Fuchs, S.; Isojärvi, J.; Vähäkangas, K. Improved detection and determination of carbamazepine and oxcarbazepine and their metabolites by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *673*, 97–105

SAMPLE**Matrix:** blood**Sample preparation:** 50 μL Plasma + 250 μL 3 $\mu\text{g}/\text{mL}$ methyl p-hydroxybenzoate in MeCN, mix, centrifuge at 10000 rpm for 5 min, inject a 10 μL aliquot of the supernatant.**HPLC VARIABLES****Column:** 150 \times 16 5 μm Cosmosil 5C8 (Shimadzu)**Mobile phase:** MeCN:5 mM KH_2PO_4 31:69**Flow rate:** 1**Injection volume:** 10**Detector:** UV 210

CHROMATOGRAM**Internal standard:** methyl p-hydroxybenzoate**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCEShinoda, M.; Akita, M.; Hasegawa, M.; Nadai, M.; Hasegawa, T.; Nabeshima, T. Pharmaceutical evaluation of carbamazepine suppositories in rats. *Biol.Pharm.Bull.*, **1995**, *18*, 1289–1291

SAMPLE**Matrix:** blood**Sample preparation:** 50 μ L Plasma + 100 μ L MeCN, centrifuge, inject a 5 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** 100 \times 4.6 3.5 μ m Zorbax SB**Mobile phase:** MeCN:MeOH:10 mM pH 7.1 phosphate buffer 7:34:59**Flow rate:** 1.5**Injection volume:** 5**Detector:** UV 220

CHROMATOGRAM**Retention time:** 7.4**Limit of detection:** <1 μ M

OTHER SUBSTANCES**Extracted:** metabolites, carbamazepine epoxide, hydroxycarbamazepine, lamotrigine (UV 310), oxcarbazepine, phenobarbital, phenytoin**Also analyzed:** ibuprofen, naproxen, trimethoprim

KEY WORDS

plasma

REFERENCESvensson, J.O. Simple HPLC method for determination of antiepileptic drugs in plasma (Abstract 102). *Ther.Drug Monit.*, **1995**, *17*, 408

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m 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: 3.67

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbinoxamine, carpipramine, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, temoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thio-pental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorbucine

Interfering: bromazepam, carbamazepine, carteolol, dihydralazine, nadolol, nalbuphine, omeprazole, procainamide, procabazepine, sotalol, strychnine

KEY WORDS

whole blood; plasma

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 μ L Serum + 600 μ L allobarbitol in 75 mM pH 6.8 phosphate buffer, add 200 units β -glucuronidase, heat at 37° for 30 min, add 1 mL of this solution

to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 2.5 mL MTBE. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L MeOH:water 50:50, inject a 10 μ L aliquot onto columns A and B in series with mobile phase A. After 12 min elute column A with mobile phase B, continue to elute column B with mobile phase A. Carbamazepine diol, carbamazepine epoxide, phenytoin, and carbamazepine elute from column A and the enantiomers of 5-(p-hydroxyphenyl)-5-phenylhydantoin and mephobarbital, phenobarbital, zonisamide, and allobarbitol elute from column B. Re-equilibrate columns A and B with mobile phase A for 5 min before the next injection.

HPLC VARIABLES

Column: A 250 \times 4 4 μ m Superspher RP-18e (E. Merck); B 250 \times 4 4 μ m Superspher RP-18e (E. Merck)

Mobile phase: A MeOH:11.2 mM β -cyclodextrin in 20 mM KH_2PO_4 5:95; B MeCN:20 mM KH_2PO_4 16:84

Flow rate: 0.8

Injection volume: 10

Detector: UV 210 (A); UV 210 (B)

CHROMATOGRAM

Retention time: 24 (column A)

Internal standard: allobarbitol

Limit of detection: 2.2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine diol, carbamazepine epoxide, 5-(p-hydroxyphenyl)-5-phenylhydantoin, mephobarbital, phenobarbital, phenytoin, zonisamide

KEY WORDS

serum; column-switching; SPE; chiral

REFERENCE

Eto, S.; Noda, H.; Noda, A. Simultaneous determination of antiepileptic drugs and their metabolites, including chiral compounds, via β -cyclodextrin inclusion complexes by a column-switching chromatographic technique. *J.Chromatogr.B*, **1994**, *658*, 385-390

SAMPLE

Matrix: blood

Sample preparation: Inject sample onto column A with mobile phase A and elute for 3 min. Backflush contents of column A onto column B with mobile phase B for 6 min and elute column B with mobile phase B and monitor eluant.

HPLC VARIABLES

Column: A 10 \times 3 BioTrap Acid C18 (ChromTech); B 10 \times 3 CT-sil C18 guard column + 150 \times 4.6 5 μ m CT-sil C18 (ChromTech)

Mobile phase: A 82 mM pH 6.0 phosphate buffer; B MeCN:82 mM pH 6.0 phosphate buffer 50:50

Flow rate: A 0.55; B 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 13

Limit of quantitation: 2050 ng/mL

OTHER SUBSTANCES

Simultaneous: phenytoin

KEY WORDS

plasma; column-switching; direct injection

REFERENCE

Hermansson, J.; Grahn, A. Determination of drugs by direct injection of plasma into a biocompatible extraction column based on a protein-entrapped hydrophobic phase. *J.Chromatogr.A*, **1994**, *660*, 119-129

SAMPLE

Matrix: blood

Sample preparation: Condition an Extrashot-Silica (diatomaceous earth) SPE cartridge (Kusano Scientific) with 200 μ L EtOH and 200 μ L dichloromethane, force out the remaining solvent with 500 μ L air. Add 5 μ L serum to the surface of the cartridge and pass 130 μ L dichloromethane gently through the cartridge into the 100 μ L sample loop.

HPLC VARIABLES

Column: 125 \times 4.5 μ m LiChrosorb Si60

Mobile phase: n-Hexane:dichloromethane:EtOH:acetic acid 82.8:15:2:0.2

Flow rate: 1

Injection volume: 100

Detector: UV 240

CHROMATOGRAM

Retention time: 14.7

Limit of quantitation: 1 μ g/mL

OTHER SUBSTANCES

Extracted: phenobarbital, phenytoin

KEY WORDS

serum; normal phase; SPE

REFERENCE

Kouno, Y.; Ishikura, C.; Homma, M.; Oka, K. Simple and accurate high-performance liquid chromatographic method for the measurement of three antiepileptics in therapeutic drug monitoring. *J.Chromatogr.*, **1993**, *622*, 47-52

SAMPLE

Matrix: blood

Sample preparation: 400 μ L Serum + 50 μ L 100 μ g/mL IS in water, mix, add 400 μ L 1.5 M NaOH, add 100 mg NaCl, add 4 mL ethyl acetate:chloroform 50:50, shake for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μ L hexane and 200 μ L mobile phase, mix, inject a 20 μ L aliquot of the mobile phase layer.

HPLC VARIABLES

Guard column: 10 \times 4.5 μ m Spherisorb ODS-2

Column: 250 \times 4.5 μ m Spherisorb ODS-2

Mobile phase: MeCN:water 30:70

Flow rate: 1.1

Injection volume: 20

Detector: UV 210

CHROMATOGRAM

Retention time: 10.9

Internal standard: 1,3-dimethyl-7-benzylxanthine (8.2) (synthesized from theophylline, bromobenzene, and potassium carbonate in boiling benzene)

Limit of detection: 80 ng/mL

Limit of quantitation: 270 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine epoxide, phenytoin

KEY WORDS

serum

REFERENCE

Martens, J.; Banditt, P. Validation of the analysis of carbamazepine and its 10,11-epoxide metabolite by high-performance liquid chromatography from plasma: comparison with gas chromatography and the enzyme-multiplied immunoassay technique. *J.Chromatogr.*, **1993**, *620*, 169–173

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 200 μ L 20 μ g/mL butalbital in MeCN, vortex, centrifuge 5 min, inject supernatant

HPLC VARIABLES

Column: 125 \times 4 LiChroSpher RP-8 5 μ m

Mobile phase: MeCN:water:100 mM pH 7.0 phosphate buffer 20:75:5

Column temperature: 45

Flow rate: 2

Injection volume: 50

Detector: UV 212

CHROMATOGRAM

Retention time: 9.4

Internal standard: butalbital (3.8)

OTHER SUBSTANCES

Simultaneous: phenobarbital, phenytoin

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: Inject 10 μ L serum onto column A with mobile phase A, after 2 min backflush contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A μ Bondapak phenyl Guard Pak; B 150 \times 3.9 μ Bondapak phenyl

Mobile phase: A 5 mM sodium dodecyl sulfate in water; B MeOH:water 30:70 containing 50 mM sodium dodecyl sulfate

Flow rate: 2

Injection volume: 10

Detector: UV 214

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine epoxide

KEY WORDS

serum; column-switching; cow

REFERENCE

Bentrop, D.; Warren, F.V., Jr.; Schmitz, S.; Bidlingmeyer, B.A. Analysis of carbamazepine in serum by liquid chromatography with direct sample injection and surfactant-containing eluents. *J.Chromatogr.*, **1990**, *535*, 293-304

SAMPLE

Matrix: blood

Sample preparation: 250 μ L Plasma + 2 μ g 10-methoxycarbamazepine + 25 μ L 1 M NaOH + 1.2 mL dichloromethane, mix for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 20 μ L MeCN, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.9 10 μ m LiChrosorb RP8

Mobile phase: MeCN:water 32:68

Flow rate: 1.8

Injection volume: 10

Detector: UV 215

CHROMATOGRAM

Retention time: 7.0

Internal standard: 10-methoxycarbamazepine (9.3)

OTHER SUBSTANCES

Extracted: oxcarbazepine, phenobarbital, primidone

Noninterfering: clobazam, clonazepam, diazepam, ethosuximide, phenytoin, valproic acid

KEY WORDS

plasma

REFERENCE

Elyas, A.A.; Goldberg, V.D.; Patsalos, P.N. Simple and rapid micro-analytical high-performance liquid chromatographic technique for the assay of oxcarbazepine and its primary active metabolite 10-hydroxycarbamazepine. *J.Chromatogr.*, **1990**, *528*, 473-479

SAMPLE

Matrix: blood

Sample preparation: Inject 20 μ L serum onto column A with mobile phase A and elute to waste, after 1.5 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 5 μm Nucleosil C18

Mobile phase: A 14 mM NaH₂PO₄ containing 6 mM Na₂HPO₄; B MeCN:MeOH:14 mM NaH₂PO₄ containing 6 mM Na₂HPO₄ 15:20:65

Flow rate: 0.8

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 30

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: phenobarbital, phenytoin, primidone

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: 500 μL Plasma + 500 μL 1 M pH 5.0 sodium acetate buffer + 50 μL 50 μg/mL cyheptamide in MeOH, vortex for 15 s, add 4 mL dichloromethane:ethyl acetate 2:1, shake for 5 min, repeat extraction. Combine the organic layers and evaporate them 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: 250 × 4.6 5 μm Spherisorb Octyl C8

Mobile phase: MeOH:MeCN:THF:10 mM pH 6.5 ammonium phosphate buffer 16:11:7:66

Column temperature: 40

Flow rate: 1.5

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 8.3

Internal standard: cyheptamide (12.6)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, carbamazepine-10,11-epoxide, carbamazepinediol, felbamate, 5-(p-hydroxyphenyl)-5-phenylhydantoin, phenytoin

Also analyzed: ethosuximide, ethotoin, lorazepam, phenobarbital, phenylethylmalonamide, primidone

KEY WORDS

plasma

REFERENCE

Rommel, R.P.; Miller, S.A.; Graves, N.M. Simultaneous assay of felbamate plus carbamazepine, phenytoin, and their metabolites by liquid chromatography with mobile phase optimization. *Ther. Drug Monit.*, **1990**, *12*, 90-96

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 μ L plasma then 50 μ L 10 μ g/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 μ L mobile phase, inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil-LC-8

Mobile phase: MeCN:water 20:80

Flow rate: 3.3

Injection volume: 15

Detector: UV 208

CHROMATOGRAM

Retention time: 11.37

Internal standard: tolylphenobarbital (7.57)

Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: amobarbital, barbital, butabarbital, caffeine, carbamazepinediol, carbamazepine epoxide, chloramphenicol, ethosuximide, glutethimide, mephenytoin, methaqualone, methyprylon, nirvanol, pentobarbital, phenacetamide, phenobarbital, phenytoin, primidone, secobarbital, theophylline

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, p-hydroxyphenobarbital, imipramine, lidocaine, methotrexate, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, vancomycin

KEY WORDS

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 μ L Serum + 100 μ L 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 μ L mobile phase, inject a 6-10 μ L 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 \times 4.6 Supelguard LC-1 (Supelco)

Column: 250 \times 4.6 5 μ m 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: 6.68

Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental

Simultaneous: 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: Add 20 μL 30 $\mu\text{g}/\text{mL}$ cyheptamide in MeOH to a tube and evaporate the MeOH, add 1 mL plasma, add 2 mL buffer, add 20 mL chloroform:t-butyl alcohol 95:5, shake horizontally at 180 cycles/min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 65°, reconstitute the residue in 50 μL mobile phase, vortex, inject a 10 μL aliquot. (Buffer was 50 mL 200 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ + 6.3 mL 400 mM NaOH, pH 11.2.)

HPLC VARIABLES

Column: 200 \times 2.1 5 μm ODS C18 (Hewlett-Packard)

Mobile phase: MeOH:water 45:55

Column temperature: 36

Flow rate: 0.5

Injection volume: 10

Detector: UV 212

CHROMATOGRAM

Retention time: 5.78

Internal standard: cyheptamide (8.92)

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine diol, carbamazepine epoxide

Noninterfering: clonazepam, phenobarbital, phenytoin

KEY WORDS

plasma; microbore

REFERENCE

Riad, L.E.; Sawchuk, R.J. Simultaneous determination of carbamazepine and its epoxide and transdiol metabolites in plasma by microbore liquid chromatography. *Clin.Chem.*, **1988**, *34*, 1863–1866

SAMPLE**Matrix:** blood

Sample preparation: 100 μL Plasma + 200 μL 1 M HCl saturated with ammonium sulfate, vortex for 20 s, add 60 μL 10 $\mu\text{g}/\text{mL}$ 4-methylprimidone in MeCN, vortex for 20 s, centrifuge at 2700 g for 5 min, inject a 5-10 μL aliquot of the MeCN layer.

HPLC VARIABLES**Column:** 250 \times 4.5 μm LiChrosorb RP-18**Mobile phase:** MeOH:THF:50 mM pH 5.9 phosphate buffer 44:1:55**Column temperature:** 50**Flow rate:** 1.1**Injection volume:** 5-10**Detector:** UV 210**CHROMATOGRAM****Retention time:** 10**Internal standard:** 4-methylprimidone (5)**Limit of detection:** 50 ng/mL**OTHER SUBSTANCES****Extracted:** phenobarbital, phenytoin, primidone, valproic acid

Simultaneous: metabolites, acetaminophen, caffeine, chloramphenicol, diazepam, ethosuximide, ethylphenylmalonamide, glutethimide, lidocaine, methylphenobarbital, pentobarbital, salicylic acid, theophylline

KEY WORDS

plasma

REFERENCE

Kushida, K.; Ishizaki, T. Concurrent determination of valproic acid with other antiepileptic drugs by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, *338*, 131–139

SAMPLE**Matrix:** blood

Sample preparation: 50 μL Serum + 50 μL 10 $\mu\text{g}/\text{mL}$ IS in MeCN, vortex for 10 s, centrifuge at 3000 g for 1 min, remove the supernatant and place it in another tube, centrifuge for 1 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES**Column:** 100 \times 8.5 μm Nova Pak C18 Radial pak

Mobile phase: MeCN:MeOH:acetone:buffer 8:21:10:61 adjusted to pH 7.95 \pm 0.02 with NaOH (Buffer was 1.36 g/L KH_2PO_4 .)

Flow rate: 2.8**Injection volume:** 20**Detector:** UV 200**CHROMATOGRAM****Retention time:** 6.44

Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (4.89)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, ethosuximide, primidone, phenobarbital, phenytoin

Simultaneous: acetaminophen, N-acetylprocainamide, aspirin, ampicillin, caffeine, cephalirin, chloramphenicol, digoxin, disopyramide, hexobarbital, indomethacin, lidocaine, mephobarbital, methsuximide, nafcillin, pentobarbital, phenylethylmalonamide, procainamide, quinidine, salicylic acid, secobarbital, sulfamerazine, sulfamethazine, terbutaline, tetracycline, theobromine, theophylline

Noninterfering: acetazolamide, amikacin, cephalosporin C, gentamicin, propranolol, sulfadiazine, sulfamethoxazole, sulfisoxazole, tobramycin, valproic acid, verapamil

KEY WORDS

serum

REFERENCE

Ou, C.-N.; Rognerud, C.L. Simultaneous measurement of ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine, and their bioactive metabolites by liquid chromatography. *Clin.Chem.*, **1984**, *30*, 1667-1670

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 50 μ L 7 μ g/mL IS in water + 1 mL buffer, vortex for 10 s, add 5 mL n-hexane:ether:n-propanol 49:49:2, shake gently for 20 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μ L mobile phase, inject a 50-100 μ L aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Partisil 5 ODS-3

Mobile phase: MeCN:buffer 28:72 (Buffer was 300 μ L 1 M KH_2PO_4 and 50 μ L 900 mM phosphoric acid in 1.8 L water, pH 4.4.)

Column temperature: 50

Flow rate: 2.8

Injection volume: 50-100

Detector: UV 195

CHROMATOGRAM

Retention time: 7.8

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)

OTHER SUBSTANCES

Extracted: ethosuximide, secobarbital

Simultaneous: mephobarbital, paramethadione, phenobarbital, primidone

Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid

Interfering: phenytoin

KEY WORDS

serum

REFERENCE

Levine, H.L.; Cohen, M.E.; Duffner, P.K.; Kustas, K.A.; Shen, D.D. An improved high-pressure liquid chromatographic assay for secobarbital in serum. *J.Pharm.Sci.*, **1982**, *71*, 1281-1283

SAMPLE**Matrix:** blood**Sample preparation:** 400 μ L Serum or plasma + 400 μ L 10 μ g/mL IS in acetone, vortex for 10 s, centrifuge at 4500-5000 g for 1 min, remove the supernatant to another tube, centrifuge for 30 s, inject a 5-7.5 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 μ Bondapak C18**Mobile phase:** MeCN:MeOH:buffer 17:28:55, final pH 6.8-7.0 (Buffer was 400 μ L 1 M KH_2PO_4 in 1 L water, pH adjusted to 6.0 with 900 mM phosphoric acid.)**Column temperature:** 30**Flow rate:** 0.7**Injection volume:** 5-7.5**Detector:** UV 195

CHROMATOGRAM**Retention time:** 18.6**Internal standard:** tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (13.8)

OTHER SUBSTANCES**Extracted:** N-desmethylnmethsuximide, ethosuximide, phenobarbital, phenytoin, primidone**Simultaneous:** acetaminophen, butalbital, caffeine, hexobarbital, methsuximide, phenacetin, phenylethylmalonamide, salicylic acid

KEY WORDS

serum; plasma

REFERENCESzabo, G.K.; Browne, T.R. Improved isocratic liquid-chromatographic simultaneous measurement of phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, and N-desmethylnmethsuximide in serum. *Clin.Chem.*, **1982**, *28*, 100-104

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Plasma + 100 μ L heptabarbital in MeOH + 500 μ L 400 mM pH 7.0 sodium phosphate buffer + 10 mL ethyl acetate, extract. Evaporate the extract to dryness at 50°, reconstitute the residue in 20 μ L MeOH, inject a 3 μ L aliquot.

HPLC VARIABLES**Guard column:** 50 \times 2.1 Whatman Co:Pell ODS**Column:** 125 \times 4.5 5 μ m SAS Hypersil**Mobile phase:** MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 7.5 with phosphoric acid.)**Flow rate:** 1.6**Injection volume:** 3**Detector:** UV 200

CHROMATOGRAM**Retention time:** 16.4**Internal standard:** heptabarbital (9.8)**Limit of quantitation:** 3.8 μ M

OTHER SUBSTANCES**Extracted:** ethosuximide, primidone, pheneturide, phenobarbital, phenytoin

Simultaneous: amobarbital, barbital, butobarbital, cyclobarbital, ethotoin, ethylphenacetamide, glutethimide, methsuximide, pentobarbital, phenylethylmalonamide, sulfamethoxazole, sulthiame

Interfering: secobarbital

KEY WORDS

plasma; horse

REFERENCE

Christofides, J.A.; Fry, D.E. Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin.Chem.*, **1980**, *26*, 499-501

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum or plasma + 200 μ L 20 μ g/mL IS in MeOH:water 10:90 + 75 μ L glacial acetic acid, vortex for 30 s, add 5 mL chloroform, shake for 5 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 200 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 2.1 Permaphase ETH (DuPont)

Column: 250 \times 4.6 CLC 1 C8 (DuPont)

Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM KH_2PO_4 and 1 mM K_2HPO_4 adjusted to pH 5.6.)

Column temperature: 25

Flow rate: 2

Injection volume: 40

Detector: UV 220

CHROMATOGRAM

Retention time: 5.9

Internal standard: alphenal (5-allyl-5-phenylbarbituric acid) (4.4)

Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Extracted: ethosuximide, phenytoin, primidone, phenobarbital

Simultaneous: amobarbital, barbital, chlordiazepoxide, codeine, cortisol, ethotoin, glutethimide, hexobarbital, mephentoin, mephobarbital, metharbital, methsuximide, nitrazepam, pentobarbital, phenacetin, secobarbital

Noninterfering: acetaminophen, acetazolamide, amphetamine, bilirubin, caffeine, diazepam, dimenhydrinate, meperidine, meprobamate, methamphetamine, methaqualone, methylphenidate, nicotine, propoxyphene, theophylline, valproate

Interfering: phensuximide

KEY WORDS

serum; plasma

REFERENCE

Rydzewski, R.S.; Gadsden, R.H.; Phelps, C.A. Simultaneous rapid HPLC determination of anticonvulsant drugs in plasma and correlation with EMIT. *Ann.Clin.Lab.Sci.*, **1980**, *10*, 89-94

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μ L Serum, plasma, or CSF + 300 μ L reagent. Flush column A to waste with 500 μ L 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 \times 2.1 40 μm preparative grade C18 (Analytichem); B 250 \times 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: 11.66

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

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

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, CSF, gastric fluid, 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 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: 11.7

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, 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, dialysate

Sample preparation: Dialyze 400 μ L plasma against 175 μ L acceptor solution through a Cuprophane membrane (15 kDa cut-off) at 37° for 10 min, inject 500 μ L acceptor solution (including the portion used for dialysis) onto column A at 0.71 mL/min, elute the contents of column A onto column B with mobile phase, remove column A from circuit and condition it with 1 mL acceptor solution, elute column B with mobile phase and monitor the effluent. Flush acceptor channel with 5 mL acceptor solution and plasma channel with 8 mL acceptor solution containing 25 μ g/mL Triton X-100. (Acceptor solution contained 5.9 g NaCl, 4.1 g sodium acetate, 0.3 g KCl, and 1.65 g sodium citrate in 1 L water, adjusted to pH 7.4 with citric acid.)

HPLC VARIABLES

Column: A 5 \times 1.6 Hypersil ODS-2; B 100 \times 3 5 μ m Spherisorb ODS-2

Mobile phase: MeCN:THF:20 mM pH 6.0 phosphate buffer 22:6.5:71.5

Column temperature: 37

Flow rate: 0.6

Detector: UV 240

CHROMATOGRAM

Retention time: 4

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: phenobarbital, phenytoin

KEY WORDS

plasma; column-switching; dialysis

REFERENCE

Johansen, K.; Krogh, M.; Andresen, A.T.; Christophersen, A.S.; Lehne, G.; Rasmussen, K.E. Automated analysis of free and total concentrations of three antiepileptic drugs in plasma with on-line dialysis and high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *669*, 281–288

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μ L 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 \times 4 30 μm LiChrocart Aluspher RP-select B (Merck)

Column: 125 \times 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; UV 254

CHROMATOGRAM

Retention time: 12.5

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, chlordiazepoxide, 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, methyl dopa, 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, tripeleppamine, 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, urine

Sample preparation: Serum. 100 μL Serum + 200 μL MeCN, vortex for 10 s, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Saliva. 250 μL Saliva + 50 μL MeCN, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Urine. Condition a Sep-Pak SPE cartridge with 5 mL MeCN then 20 mL water. Add 2 mL urine to the cartridge, wash with 20 mL water, elute with 500 μL MeCN, inject 2 μL of the eluent.

HPLC VARIABLES

Guard column: 20 \times 2 3 μm ODS-Hypersil

Column: 250 \times 2 3 μm ODS-Hypersil

Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110

Column temperature: 40

Flow rate: 0.2

Injection volume: 2

Detector: UV 200

CHROMATOGRAM

Retention time: 13.1

Limit of quantitation: 780 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine-10,11-epoxide, clonazepam, dihydrodihydroxycarbamazepine, hexobarbital, p-hydroxyphenobarbital, 5-(m-hydroxyphenyl)-5-phenylhydantoin, 5-(p-hydroxyphenyl)-5-phenylhydantoin, nitrazepam, phenobarbital, phenylethylmaleimide, phenytoin, primidone

Noninterfering: chlordiazepoxide, cyheptamide, diazepam, lorazepam, nordiazepam, oxazepam, prazepam, temazepam

KEY WORDS

serum; SPE

REFERENCE

Liu, H.; Delgado, M.; Forman, L.J.; Eggers, C.M.; Montoya, J.L. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. *J. Chromatogr.*, **1993**, *616*, 105-115

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize 20-200 mg brain tissue with 1 mL 1.5 µg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1, flush apparatus with 1 mL extraction buffer, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 µL MeOH, add 50 µL water, inject a 10-25 µL aliquot. Serum. 100 µL Serum + 1 mL 1.5 µg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1, mix, add 1 mL extraction buffer, mix, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 µL MeOH, add 50 µL water, inject a 10-25 µL aliquot. (Extraction buffer was 20 g NaH₂PO₄·2H₂O + 4.5 g Na₂HPO₄·2H₂O + 1.5 g NaN₃ in 1 L water, pH 6. Extraction solvent was dichloromethane:isopropanol 97:3.)

HPLC VARIABLES

Column: 200 × 2.1 5 µm Hypersil ODS

Mobile phase: Gradient. A was MeCN:50 mM (NH₄)₂PO₄ (pH 4.4) 10:90. B was MeCN:50 mM (NH₄)₂PO₄ (pH 4.4) 60:40. A:B from 85:15 to 55:45 over 9.5 min, keep at 55:45 for 0.5 min, return to 85:15 over 0.5 min.

Column temperature: 65

Flow rate: 0.3

Injection volume: 10-25

Detector: UV 207

CHROMATOGRAM

Retention time: 10.63

Internal standard: 5-ethyl-5-(p-tolyl)barbituric acid (9.07)

Limit of quantitation: 150 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine-10,11-epoxide, N-desmethylnmethsuximide, phenobarbital, phenytoin, primidone

KEY WORDS

serum; SPE; brain

REFERENCE

Juergens, U.; Rambeck, B. Sensitive analysis of antiepileptic drugs in very small portions of human brain by microbore HPLC. *J.Liq.Chromatogr.*, **1987**, *10*, 1847-1863

SAMPLE**Matrix:** dialysate**Sample preparation:** Inject a 25 μ L aliquot of dialysate containing 1 μ g/mL IS.

HPLC VARIABLES**Guard column:** 30 \times 2.1 5 μ m Spheri-5 ODS**Column:** 220 \times 2.1 5 μ m Spheri-5 ODS**Mobile phase:** MeCN:buffer 27:73 (After 8 min increase flow to 0.5 mL/min over 2 min, maintain at 0.5 mL/min for 12 min, return to 0.2 mL/min over 3 min. Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 5 M NaOH.)**Flow rate:** 0.2**Injection volume:** 25**Detector:** UV 212

CHROMATOGRAM**Internal standard:** 2-methyl-5H-dibenz(b,f)azepine-5-carboxamide**Limit of detection:** 2.5 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDS

pharmacokinetics; narrow bore; rat

REFERENCE

Van Belle, K.; Sarre, S.; Ebinger, G.; Michotte, Y. Brain, liver and blood distribution kinetics of carbamazepine and its metabolic interaction with clomipramine in rats: A quantitative microdialysis study. *J.Pharmacol.Exp.Ther.*, **1995**, *272*, 1217-1222

SAMPLE**Matrix:** dialysate**Sample preparation:** Inject a 10 μ L aliquot of the dialysate.

HPLC VARIABLES**Column:** 100 \times 4.6 3 μ m Econosphere C18**Mobile phase:** MeOH:MeCN:water 17:23:60**Column temperature:** 40**Flow rate:** 0.6**Injection volume:** 10**Detector:** UV 210

CHROMATOGRAM**Retention time:** 7.55**Limit of quantitation:** 20 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites, carbamazepine-10,11-epoxide, carbamazepine-10,11-trans-diol

KEY WORDS

brain

REFERENCE

Scheyer, R.D.; During, M.J.; Cramer, J.A.; Toftness, B.R.; Hochholzer, J.M.; Mattson, R.H. Simultaneous HPLC analysis of carbamazepine and carbamazepine epoxide in human brain microdialysate. *J.Liq.Chromatogr.*, **1994**, *17*, 1567–1576

SAMPLE**Matrix:** formulations**Sample preparation:** 100 μ L Solution + 100 μ L IS solution, make up to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Spherisorb ODS-II**Mobile phase:** MeOH:water 60:40**Flow rate:** 1**Detector:** UV 212

CHROMATOGRAM**Retention time:** 4.3**Internal standard:** carbamazepine 10,11-epoxide (6.6)

KEY WORDS

suspensions; saline; 5% dextrose

REFERENCE

Clark-Schmidt, A.L.; Garnett, W.R.; Lowe, D.R.; Karnes, H.T. Loss of carbamazepine suspension through nasogastric feeding tubes. *Am.J.Hosp.Pharm.*, **1990**, *47*, 2034–2037

SAMPLE**Matrix:** formulations**Sample preparation:** Finely powder tablets, weigh out amount equivalent to 30 mg guanabenz acetate, add 190 mL MeCN, sonicate for a few minutes, make up to 200 mL with MeCN, filter. Remove a 14-59 mL aliquot and add it to 9 mL 1 mg/mL carbamazepine in MeCN, make up to 100 mL with MeCN, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 10 μ m octadecylsilane (Perkin-Elmer)**Mobile phase:** MeCN:buffer 35:65 (Buffer was 4 mM KH_2PO_4 adjusted to pH 3.25 with phosphoric acid.)**Flow rate:** 2.5**Injection volume:** 20**Detector:** UV 265

CHROMATOGRAM**Retention time:** 3**Internal standard:** carbamazepine

OTHER SUBSTANCES**Simultaneous:** guanabenz, mefruside

KEY WORDS

tablets; carbamazepine is IS

REFERENCE

Vio, L.; Mamolo, M.G.; Furlan, G. Quantitative high pressure liquid chromatographic determination of guanabenz and mephroside in pharmaceutical formulations. *Farmaco.[Prat.]*, **1988**, *43*, 27-36

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: 6.15 (A), 5.49 (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, cetirizine, chlorcyclizine, chlor-diazepoxide, 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, fenopropfen, 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, mephobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, pimozone, 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, sulfapyrazone, 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: 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 KH₂PO₄ and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 208; UV 233

CHROMATOGRAM

Retention time: 4.2

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, chlordiazepoxide, 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

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, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, 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, dapson, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, di-

prenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, 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, isocarboxystyryl, 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, mephenetermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methylidopa, methylidopamine, methylphenidate, methylprednisolone, methyltestosterone, methylprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, pyrihydione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, 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 Econosil C8

Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH₂PO₄ 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: amitriptyline, amoxapine, imipramine, nortriptyline

Also analyzed: cyclobenzaprine, desipramine, doxepin, maprotiline, protriptyline

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: 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 μ L 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 \times 4.5 μ m Hypersil octadecylsilica ODS; B 200 \times 4.6 5 μ m 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 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 16.2

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, 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

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Liu, H.; Delgado, N.R. Improved therapeutic monitoring of drug interactions in epileptic children using carbamazepine polytherapy. *Ther Drug Monit.*, **1994**, *16*, 132–138 [column temp 40; simultaneous metabolites]

Ramachandran, S.; Underhill, S.; Jones, S.R. Measurement of lamotrigine under conditions measuring phenobarbitone, phenytoin, and carbamazepine using reversed-phase high-performance liquid chromatography at dual wavelengths. *Ther Drug Monit.*, **1994**, *16*, 75–82 [serum;LOD 200 ng/mL;extracted lamotrigine, phenobarbital, phenytoin;hexabarbital (IS);non-interfering ethosuximide, oxcarbazepine, primidone, valproic acid]

Rambeck, B.; May, T.W.; Jurgens, M.U.; Blankenhorn, V.; Jurges, U.; Korn-Merker, E.; Salke-Kellermann, A. Comparison of phenytoin and carbamazepine serum concentrations measured by high-

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- Bonato, P.S.; Lanchote, V.L. A rapid procedure for the purification of biological samples to be analysed by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1993**, *16*, 2299–2308 [also alben-dazole, clonazepam, desalkylflurazepam, mebendazole; methaqualone (IS)]
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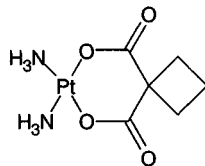
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Carboplatin

Molecular formula: C₆H₁₂N₂O₄Pt

Molecular weight: 371.3

CAS Registry No.: 41575-94-4



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 500 μ L MeCN:MeOH:water 10:40:50, vortex, filter through a Centricon-10 10000 molecular mass cut-off filter (Amicon) with centrifuging at 2677 g for 30 min, inject a 10-30 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Spherisorb phenyl

Mobile phase: MeOH:water 5:95 for 5 min then MeCN:MeOH:isopropanol:water 45:45:5:5 at 2.5 mL/min, re-equilibrate with MeOH:water 5:95 for 10 min

Column temperature: 50

Flow rate: 0.5

Injection volume: 10-30

Detector: UV 210

CHROMATOGRAM

Retention time: 3.6

Limit of detection: 10 ppb (100 μ L injection)

KEY WORDS

plasma; dog; ultrafiltrate

REFERENCE

Tyczkowska, K.; Page, R.L.; Riviere, J.E. Determination of carboplatin in canine plasma by liquid chromatography with ultraviolet-visible detection and confirmation by atomic absorption spectroscopy. *J.Chromatogr.*, **1990**, 527, 447-453

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: 250 \times 4.6 Inertsil ODS-2

Mobile phase: MeCN:10 mM pH 5.5 buffer 5:95

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 and the mixture flowed through a 10 m \times 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: 6.5

Limit of detection: 60 nM

OTHER SUBSTANCES

Simultaneous: oxaliplatin, tetraplatin

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: 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: formulations

Sample preparation: Emulsion. 500 μL Emulsion + 10 mL 400 μg/mL hydroquinone in MeOH + 40 mL Tween 80, shake until homogeneous, inject a 10 μL aliquot. Drug release medium. 1 mL Drug release medium + 200 μL 100 μg/mL hydroquinone, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Cosmosil 10 C18 (Nacalai Tesque)

Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 2:98 for 1 min, to 45:55 over 5.5 min, maintain at 45:55 for 2 min, return to initial conditions over 1 min.

Flow rate: 2

Injection volume: 10-50

Detector: UV 220

CHROMATOGRAM

Retention time: 2.2

Internal standard: hydroquinone (4.2)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: epirubicin, mitomycin C, iomeprol

KEY WORDS

emulsions; drug release medium; injections

REFERENCE

Yamazoe, K.; Horiuchi, T.; Sugiyama, T.; Katagiri, Y. Simultaneous high-performance liquid chromatographic determination of carboplatin, epirubicin hydrochloride and mitomycin C in a Lipiodol emulsion. *J.Chromatogr.A*, **1996**, 726, 241–245

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:** 3**Limit of detection:** 1 μg/mL**Limit of quantitation:** 5 μg/mL**OTHER SUBSTANCES****Simultaneous:** cisplatin**KEY WORDS**

infusion fluids; 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

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Prat, J.; Pujol, M.; Girona, V.; Munoz, M.; Sole, L.A. Stability of carboplatin in 5% glucose solution in glass, polyethylene and polypropylene containers. *J.Pharm.Biomed.Anal.*, **1994**, 12, 81–84 [stability-indicating; 5% dextrose]

Hadfield, J.A.; McGown, A.T.; Dawson, M.J.; Thatcher, N.; Fox, B.W. The suitability of carboplatin solutions for 14-day continuous infusion by ambulatory pump: an HPLC-dynamic FAB study. *J.Pharm.Biomed.Anal.*, **1993**, 11, 723–727

Allsopp, M.A.; Sewell, G.J.; Rowland, C.G. A column-switching liquid chromatography assay for the analysis of carboplatin in plasma ultrafiltrate. *J.Pharm.Biomed.Anal.*, **1992**, 10, 375–381

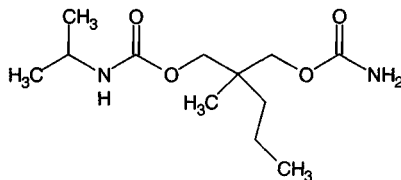
Duncan, G.F.; Faulkner, H.C.; Farnen, R.H.; Pittman, K.A. Liquid chromatographic procedure for the quantitative analysis of carboplatin in beagle dog plasma ultrafiltrate. *J.Pharm.Sci.*, **1988**, 77, 273–276

Elferink, F.; van der Vijgh, W.J.; Pinedo, H.M. On-line differential pulse polarographic detection of carboplatin in biological samples after chromatographic separation. *Anal.Chem.*, **1986**, 58, 2293–2296

Gaver, R.C.; Deeb, G. High-performance liquid chromatographic procedures for the analysis of carboplatin in human plasma and urine. *Cancer Chemother.Pharmacol.*, **1986**, 16, 201–206

Ding, X.-D.; Krull, I.S. Dual electrode liquid chromatography-electrochemical detection (LCEC) for platinum-derived cancer chemotherapy agents. *J.Liq.Chromatogr.*, **1983**, 6, 2173–2194

Carisoprodol



Molecular formula: C₁₂H₂₄N₂O₄

Molecular weight: 260.3

CAS Registry No.: 78-44-4

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 100 μ L 200 mM HCl + 1 mL chloroform, shake for 2 min, sonicate for 1 min, centrifuge for 1 min. Remove the organic layer and add it to 200 mM NaOH, shake for 1 min, sonicate for 1 min, centrifuge for 1 min. Remove 750 μ L of the organic layer and evaporate it to dryness, dissolve the residue in 50 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 3 octyl CP-tm-Spher C8 glass column (Chrompack)

Mobile phase: MeCN:50 mM NaH₂PO₄ 35:65, adjusted to pH 2.2 with phosphoric acid

Flow rate: 0.8

Injection volume: 20

Detector: UV 190

CHROMATOGRAM

Retention time: 5.5

Internal standard: carisoprodol

OTHER SUBSTANCES

Simultaneous: meprobamate

KEY WORDS

serum; carisoprodol is IS

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: solutions

Sample preparation: Inject a 40 μ L aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Porasil

Mobile phase: THF:toluene 50:50

Flow rate: 2

Injection volume: 40

Detector: RI

CHROMATOGRAM

Retention time: 2

Internal standard: acetaminophen (3)

OTHER SUBSTANCES

Simultaneous: caffeine, phenacetin

KEY WORDS

normal phase

REFERENCE

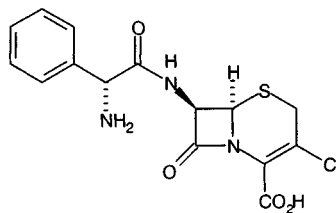
Honigberg, I.L.; Stewart, J.T.; Smith, M. Liquid chromatography in pharmaceutical analysis IX: Determination of muscle relaxant-analgesic mixtures using normal phase chromatography. *J.Pharm.Sci.*, **1978**, *67*, 675-679

Cefaclor

Molecular formula: C₁₅H₁₄ClN₃O₄S

Molecular weight: 367.8

CAS Registry No.: 53994-73-3, 70356-03-5 (monohydrate)



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 100 μ L 6 μ g/mL cefotaxime + 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 μ L aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m C18 (Waters)

Mobile phase: MeCN:100 mM phosphate 8:92, pH 5.6

Flow rate: 1.2

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Internal standard: cefotaxime

Limit of detection: 200 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

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 μ L Plasma + 25 μ L 8.5% phosphoric acid + 250 μ L 1 mg/mL coumarin-3-carboxylic acid in water, add to the C18 SPE cartridge, wash with 500 μ L 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 μ L aliquot of the eluate.

HPLC VARIABLES

Column: 250 \times 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

Internal standard: coumarin-3-carboxylic acid (13)

OTHER SUBSTANCES

Extracted: cefazolin, ceftizoxime, cephalixin

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. *Clin.Chim.Acta*, **1990**, *190*, 121–123

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 10 μL 100 $\mu\text{g}/\text{mL}$ 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: Guard Pak C18 (Waters)

Column: 200 \times 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: 5.8

Internal standard: cephradine

Limit of detection: 1 $\mu\text{g}/\text{mL}$

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 $\mu\text{g}/\text{mL}$ 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 \times 4.6 5 μm 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:** 6**Internal standard:** cefixime (11)**Limit of detection:** 1 $\mu\text{g/mL}$

OTHER SUBSTANCES**Extracted:** cefadroxil, cephalixin, cephradine**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, cephalixin, and cephradine—in human serum. *Clin.Chem.*, **1987**, *33*, 1788–1790

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

HPLC VARIABLES**Column:** 250 \times 4.6 5 μm GFF-S5-80 internal-surface reversed phase "Pinkerton" (Regis)**Mobile phase:** 100 mM pH 4.38 sodium phosphate buffer containing 20 mM sodium dodecyl sulfate**Flow rate:** 0.8**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 13

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**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 \times 4 10 μm C18**Column:** 300 \times 4 10 μm μ Bondapak C18**Mobile phase:** MeCN:MeOH:100 mM sodium acetate 8.64:0.36:91, pH 5.2**Flow rate:** 2.5**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 6**Internal standard:** 8-chlorotheophylline (8.5)**Limit of detection:** 500 ng/mL

KEY WORDSplasma

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, 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 μ L Plasma + 1 mL 1% phosphoric acid, mix, add to the SPE cartridge, wash with 3 mL 1% perchloric acid, elute with 750 μ L MeOH, inject a 50 μ L 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 μ L 10 mM pH 7.0 phosphate buffer, rotate at 4° for 12 h, centrifuge at 800 g for 10 min. 400 μ L Supernatant + 1 mL 1% phosphoric acid (?), mix, add to the SPE cartridge, wash with 1% perchloric acid, elute with 150 μ L MeOH, inject a 75 μ L aliquot of the eluate.

HPLC VARIABLES**Guard column:** 20 \times 4.6 5 μ m C18 (Shandon)**Column:** 250 \times 4.6 5 μ m 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:** 18.2**Internal standard:** cefaclor

OTHER SUBSTANCES**Extracted:** cefpodoxime

KEY WORDSplasma; cefaclor is IS; 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, urine

Sample preparation: Plasma or serum. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH then 2 mL water, do not allow to go dry. 500 μ L Plasma or serum + 100 μ L 100 μ g/mL cephalixin in water + 50 μ L 25% acetic acid, mix, add to SPE cartridge, wash with two 1 mL portions of water, elute with 3 mL MeOH. Evaporate eluate under nitrogen, add 200 μ L mobile phase, vortex, inject a 25 μ L aliquot. Urine. Dilute 100:1 (ratio may vary depending on concentration) with water, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18-DB

Mobile phase: MeOH:THF:buffer 16:4:80 (Buffer was 1 g sodium 1-heptanesulfonate + 15 mL triethylamine in 1 L water with the pH adjusted to 2.3 with concentrated phosphoric acid.)

Column temperature: 30

Flow rate: 1.4

Injection volume: 25-50

Detector: UV 265

CHROMATOGRAM

Retention time: 6

Internal standard: cephalixin (9.2)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: hydroxyloracarbef, loracarbef

Noninterfering: acetaminophen, caffeine

KEY WORDS

plasma; serum; SPE; pharmacokinetics

REFERENCE

Kovach, P.M.; Lantz, R.J.; Brier, G. High-performance liquid chromatographic determination of loracarbef, a potential metabolite, cefaclor and cephalixin in human plasma, serum and urine. *J.Chromatogr.*, **1991**, 567, 129-139

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

Retention time: 14

Limit of detection: 1.3 ng/mL (F); 1.6 ng/mL (UV)

OTHER SUBSTANCES

Also analyzed: cefroxadine, cephalixin, cephradine

Noninterfering: amidopyrin, aspirin, barbital, caffeine, cefmenoxime, cefotaxime, ceftizoxime, ceftriaxone, cetazidime, diazepam, dibekacin, gentamycin, nycin, lidocaine, netilmicin, tetracaine, theophylline, tobramycin

KEY WORDS

post-column reaction; plasma; F detection may be less susceptible to interferences

REFERENCE

Blanchine, 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

Sample preparation: Dissolve in water at a concentration of 5 mg/mL, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m YMC-ODS (YMC)

Mobile phase: Gradient. A was 50 mM sodium dihydrogen phosphate adjusted to pH 4.0 with phosphoric acid. B was MeCN:50 mM sodium dihydrogen phosphate adjusted to pH 4.0 with phosphoric acid 45:55. A:B from 95:5 to 75:25 over 30 min, to 0:100 over 15 min, maintain at 0:100 for 5 min, return to 95:5 and equilibrate for 14 min.

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 28

OTHER SUBSTANCES

Simultaneous: impurities, excipients

Also analyzed: cephalixin

REFERENCE

Olsen, B.A.; Baertschi, S.W.; Riggin, R.M. Multidimensional evaluation of impurity profiles for generic cephalixin and cefaclor antibiotics. *J.Chromatogr.*, **1993**, *648*, 165–173

SAMPLE

Matrix: bulk, formulations

Sample preparation: Dissolve in water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:water:acetic acid 30:70:0.1

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 7.5

Limit of quantitation: 2 μ g/mL

OTHER SUBSTANCES

Simultaneous: impurities, cefadroxil, cefamandole, cefamandole nafate, cefazolin, cefoperazone, cefotaxime, cefoxitin, ceftizoxime, cephalixin, cephalothin, cephapirin, cephradine

REFERENCE

Ting, S. Reverse-phase liquid chromatographic analysis of cephalosporins. *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130

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: 6.7

OTHER SUBSTANCES

Simultaneous: amoxicillin, ampicillin, cephalixin, cephradine

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: Inject 100 μL onto column A with mobile phase A, after 3 min back-flush 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: 6.71

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: cefaloridine, 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

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Muranushi, N.; Horie, K.; Masuda, K.; Hirano, K. Characteristics of ceftibuten uptake into Caco-2 cells. *Pharm.Res.*, **1994**, *11*, 1761–1765 [also cefadroxil, cefazolin, cephalixin, cephradine, cyclacillin, latamoxef]

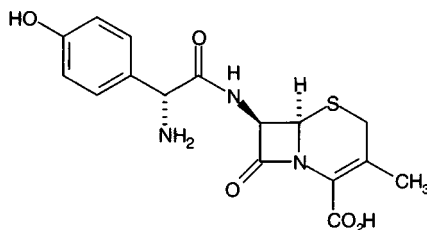
- Lorenz, L.J.; Bashore, F.N.; Olsen, B.A. Determination of process-related impurities and degradation products in cefaclor by high-performance liquid chromatography. *J.Chromatogr.Sci.*, **1992**, *30*, 211–216 determination, [simultaneous impurities, degradation products; bulk; gradient; column temp 25]
- Nahata, M.C.; Jackson, D.S. Liquid chromatographic method for the determination of cefadroxil in its suspension and in serum. *J.Liq.Chromatogr.*, **1990**, *13*, 1651–1656 [simultaneous cefadroxil; suspensions; cefaclor is IS]
- Nahata, M.C. Determination of cefaclor by high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *228*, 429–433
- Rotschafer, J.C.; Crossley, K.B.; Lesar, T.S.; Zaske, D.; Miller, K. Cefaclor pharmacokinetic parameters: serum concentrations determined by a new high-performance liquid chromatographic technique. *Antimicrob.Agents Chemother.*, **1982**, *21*, 170–172
- Ullmann, U. High-pressure liquid chromatography and microbiological assay in the determination of serum levels using cefradine and cefaclor. *Zentralbl.Bakteriol.[A]*, **1980**, *248*, 414–421

Cefadroxil

Molecular formula: C₁₆H₁₇N₃O₅S

Molecular weight: 363.4

CAS Registry No.: 66592-87-8



SAMPLE

Matrix: blood

Sample preparation: 100 μ L 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 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

OTHER SUBSTANCES

Simultaneous: antipyrine

KEY WORDS

plasma; rat; cefadroxil 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 μ L Serum + 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 μ L aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m 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

OTHER SUBSTANCES

Extracted: ceftibuten

KEY WORDS

serum; mouse; cefadroxil is IS

REFERENCE

Onyeji, C.O.; Nicolau, D.P.; Nightingale, C.H.; Quintiliani, R. Optimal times above MICs of ceftibuten and cefacor in experimental intra-abdominal infections. *Antimicrob.Agents Chemother.*, **1994**, *38*, 1112-1117

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 100 μ L 100 μ g/mL cefadroxil + 300 μ L 5% trichloroacetic acid + 500 μ L MeCN + 1.5 mL dichloromethane, vortex for 10 s, centrifuge at 500-600 g at 5° for 10 min, inject a 25 μ L aliquot of the aqueous supernatant.

HPLC VARIABLES

Guard column: 23 \times 4 37-50 μ m Corasil C18

Column: 150 \times 4 Nova-Pak

Mobile phase: MeCN:5 mM 1-octanesulfonic acid 12:88

Flow rate: 1

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 10

Internal standard: cefadroxil

OTHER SUBSTANCES

Extracted: cefepime

KEY WORDS

plasma; rat; cefadroxil is IS

REFERENCE

Barbhaiya, R.H.; Fergie, 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

Sample preparation: 100 μ L Serum + 10 μ L 200 μ g/mL cephradine in water + 100 μ L 6% trichloroacetic acid, vortex, centrifuge at 9000 g for 10 min, inject 25 μ L supernatant.

HPLC VARIABLES

Guard column: Waters Guard-Pak C18

Column: 200 \times 4.6 5 μ m 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: 7.7

Internal standard: cephradine

Limit of quantitation: 1 μ g/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 μ L Serum + 10 μ L 5 μ g/mL cefixime in MeOH + 100 μ L 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 μ L mobile phase, inject a 50-80 μ L aliquot.

HPLC VARIABLES**Guard column:** RCSS Silica Guard Pak (Waters)**Column:** 150 \times 4.6 5 μ m Ultrasphere Octyl C8**Mobile phase:** MeOH:12.5 mM pH 2.6 NaH₂PO₄ (pH adjusted with concentrated phosphoric acid) 20:80**Flow rate:** 2**Injection volume:** 50-80**Detector:** UV 240

CHROMATOGRAM**Retention time:** 3**Internal standard:** cefixime (11)**Limit of detection:** 1 μ g/ mL

OTHER SUBSTANCES**Extracted:** cefaclor, cephalixin, cephradine**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, cephalixin, and cephradine—in human serum. *Clin.Chem.*, **1987**, *33*, 1788-1790

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 1 mL MeCN, shake 30 s, centrifuge 5 min. Transfer upper layer phase, add 6 mL dichloromethane, shake 5 min, centrifuge 5 min, inject 10-100 μ L upper aqueous phase.

HPLC VARIABLES**Column:** 300 \times 3.9 μ Bondapak C18**Mobile phase:** MeOH:10 mM pH 4.8 buffer 5:95**Flow rate:** 1.5**Injection volume:** 10-100**Detector:** UV 240

CHROMATOGRAM**Retention time:** 6.4**Limit of detection:** 150 ng/mL

KEY WORDS

plasma

REFERENCE

Brisson, A.M.; Fourtillan, J.B. Pharmacokinetic study of cefadroxil following single and repeated doses. *J.Antimicrob.Chemother.*, **1982**, *10 Suppl B*, 11-15

SAMPLE

Matrix: blood, middle ear fluid

Sample preparation: Condition a 2.8 mL 500 mg Bond Elut C18 SPE cartridge with 4 mL MeOH and 1 mL 50 mM pH 6.8 phosphate buffer. 200 μ L Plasma or 50 μ L middle ear fluid + 1 mL 50 mM pH 6.8 phosphate buffer, vortex, add to the SPE cartridge, wash with 1 mL 50 mM pH 6.8 phosphate buffer, wash with 1 mL buffer, dry under vacuum, elute with 1 mL MeOH:water 40:60. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 35 μ L MeOH:water 5:95, inject a 25 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 2.5 μ m MOS Hypersil C8

Column: 150 \times 2.5 μ m MOS Hypersil C8

Mobile phase: MeCN:5 mM phosphate buffer containing 5 mM tetrabutylammonium 6:94, adjusted to pH 6.5 (After 14 min wash column with MeCN:buffer 25:75 for 2 min, re-equilibrate for 4 min.)

Column temperature: 40

Flow rate: 0.35

Injection volume: 25

Detector: UV 210

CHROMATOGRAM

Retention time: 12.30

Internal standard: cefadroxil

OTHER SUBSTANCES

Extracted: amoxicillin

KEY WORDS

plasma; SPE; cefadroxil is IS

REFERENCE

Yuan, Z.; Russlie, H.Q.; Canafax, D.M. Sensitive assay for measuring amoxicillin in human plasma and middle ear fluid using solid-phase extraction and reversed-phase high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *674*, 93-99

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 150 μ L Plasma + 150 μ L 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 \times 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: 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 5:95

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: 0.3 ng/mL (F); 0.6 ng/mL (UV)

OTHER SUBSTANCES

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

Blanchine, 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: Homogenize sample, weigh out sample equivalent to 50 mg cefadroxil, make up to 50 mL with 100 mM pH 4.5 phosphate buffer. Take 5 mL of this solution, add 0.5 mL 30 mg/mL dimethyl phthalate in MeCN:water 1:1, make up to 50 mL with 100 mM pH 4.5 phosphate buffer.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:10 mM pH 4.5 phosphate buffer 60:40

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM**Retention time:** 2**Internal standard:** dimethyl phthalate**Limit of quantitation:** 20 $\mu\text{g/mL}$

KEY WORDS

capsules; powders

REFERENCE

Hsu, M.-C.; Chang, Y.-W.; Lee, Y.-T. Column liquid chromatography and microbiological assay compared for determination of cefadroxil preparations. *J.Chromatogr.*, **1992**, 609, 181-186

SAMPLE**Matrix:** bulk, formulations**Sample preparation:** Dissolve in water to a concentration of 40 $\mu\text{g/mL}$, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$ **Mobile phase:** MeOH:water:acetic acid 30:70:0.1**Flow rate:** 1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5**Limit of quantitation:** 1.8 $\mu\text{g/mL}$

OTHER SUBSTANCES**Simultaneous:** impurities, cefaclor, cefamandole, cefamandole nafate, cefazolin, cefoperazone, cefotaxime, cefoxitin, ceftizoxime, cephalixin, cephalothin, cephapirin, cephradine

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 Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 1 mL 1 M oxalic acid, heat at 60° for 10 min, centrifuge for 10 min, remove the supernatant and add it to 20 mL water and 400 μL tributylamine, shake well, add 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 \times 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; Charm II assay

OTHER SUBSTANCES

Extracted: amoxicillin, ticarcillin

Simultaneous: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G

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: perfusate

HPLC VARIABLES

Guard column: 40 μ m C18 (Teknochroma)

Column: 150 \times 3.9 4 μ m Novapak C18 (Teknochroma)

Mobile phase: MeCN:buffer 16:84, adjusted to pH 3.9 with dilute NaOH (Buffer was 5.8 mL glacial acetic acid and 2.456 g sodium laurylsulfate in 1 L water.)

Flow rate: 1

Detector: UV 280

KEY WORDS

rat

REFERENCE

Sancho-Chust, V.; Fabra-Campos, S.; Gómez-Meseguer, V.; Bengochea, M.; Martín-Villodre, A. Experimental studies on the influence of surfactants on intestinal absorption of drugs. Cefadroxil as model drug and sodium lauryl sulfate as model surfactant: Studies in rat colon. *Arzneimittelforschung*, 1995, 45, 595–601

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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: 6.5

OTHER SUBSTANCES

Simultaneous: 7-aminocephalosporanic acid, cefazolin, cefotaxime, cephalixin, 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: surface wipes

Sample preparation: Swab 100 × 100 mm surface with water (total volume 10 mL), remove excess liquid with a second swab, vortex swabs for 45 s, filter (0.45 μm polycarbonate), inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:70 mM KH₂PO₄ 4:96

Flow rate: 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 5.6

Limit of quantitation: 100 ng/mL

REFERENCE

Gorski, R.J.; Plaszc, 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

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 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: 6.7

Internal standard: cefadroxil

OTHER SUBSTANCES

Extracted: cefepime

KEY WORDS

mouse; muscle; cefadroxil is IS

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

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Changqin, H.; Shaohong, S.; Kaimin, W. The chromatographic behavior of cephalosporins in gel filtration chromatography, a novel method to separate high molecular weight impurities. *J.Pharm.*

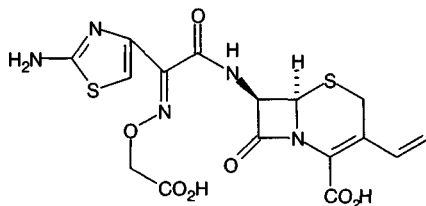
- Biomed.Anal.*, **1994**, *12*, 533–541 [also cefamandole, cefmenoxime, cefoperazone, cefotaxime, ceftazidime, ceftriaxone, cephalixin, cephaloridine, cephalothin, cephadrine]
- Muranushi, N.; Horie, K.; Masuda, K.; Hirano, K. Characteristics of ceftibuten uptake into Caco-2 cells. *Pharm.Res.*, **1994**, *11*, 1761–1765 [also cefaclor, cefazolin, ceftibuten, cephalixin, cephadrine, cyclo-cillin, latamoxef]
- Snippe, N.; Van de Merbel, N.C.; Ruiten, F.P.M.; Steijer, O.M.; Lingeman, H.; Brinkman, U.A.T. Automated column liquid chromatographic determination of amoxicillin and cefadroxil in bovine serum and muscle tissue using on-line dialysis for sample preparation. *J.Chromatogr.B*, **1994**, *662*, 61–70 [extracted amoxicillin; cow; serum; muscle; on-line dialysis; SPE; post-column reaction; LOD 50 ng/mL; LOD 200 ng/g]
- Nahata, M.C.; Jackson, D.S. Liquid chromatographic method for the determination of cefadroxil in its suspension and in serum. *J.Liq.Chromatogr.*, **1990**, *13*, 1651–1656 [suspensions; serum; cefaclor (IS)]

Cefixime

Molecular formula: C₁₆H₁₅N₅O₇S₂

Molecular weight: 453.4

CAS Registry No.: 79350-37-1



SAMPLE

Matrix: bile, blood

Sample preparation: Plasma. 25 μ L Plasma + 25 μ L buffer + 450 μ L 5% trichloroacetic acid, mix, centrifuge, inject a 10 μ L aliquot of the supernatant. Bile. 100 μ L Bile + 900 μ L buffer, mix, centrifuge at 2000 rpm for 5 min, inject a 10 μ L aliquot of the supernatant. (Buffer was 2.54% NaH₂PO₄·2H₂O and 4.41% Na₂HPO₄·12H₂O, pH 7.4.)

HPLC VARIABLES

Column: 150 \times 4.6 Chemcosorb 7C18 (Chemco)

Mobile phase: MeCN:buffer 11:89 (plasma) or 13:87 (bile) (Buffer was 20 mM (NH₄)H₂PO₄ adjusted to pH 3.2 with phosphoric acid.)

Column temperature: 40

Flow rate: 2 (plasma), 1 (bile)

Injection volume: 10

Detector: UV 290

KEY WORDS

rat; plasma

REFERENCE

Yasui, H.; Yamaoka, K.; Nakagawa, T. Alternative continuous infusion method for analysis of enterohepatic circulation and biliary excretion of cefixime in the rat. *J.Pharm.Sci.*, **1994**, *83*, 819–823

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 10 μ L 5 μ g/mL cephalexin in MeOH + 100 μ L 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 μ L mobile phase, inject a 50–80 μ L aliquot.

HPLC VARIABLES

Guard column: RCSS Silica Guard Pak (Waters)

Column: 150 \times 4.6 5 μ m Ultrasphere Octyl C8

Mobile phase: MeOH:12.5 mM pH 2.6 NaH₂PO₄ (pH adjusted with concentrated phosphoric acid) 20:80

Flow rate: 2

Injection volume: 50–80

Detector: UV 240

CHROMATOGRAM

Retention time: 11

Internal standard: cephalexin (15)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: cefaclor, cefadroxil, cephradine

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, cephalixin, and cephadrine—in human serum. *Clin.Chem.*, **1987**, *33*, 1788–1790

SAMPLE

Matrix: blood, CSF

Sample preparation: 150 μ L Serum or CSF + 150 μ L 6% trichloroacetic acid, centrifuge for 3 min, inject 75 μ L supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 3 μ m Techsphere C18

Mobile phase: 170 mL MeCN + 1.36 g NaH₂PO₄ + 2 mL phosphoric acid + 828 mL water adjusted to pH 2.7

Flow rate: 2

Injection volume: 75

Detector: UV 313

KEY WORDS

serum; method has advantages over that of Falkowski et al. (*J. Chromatogr.* 1987; 422; 145-152)

REFERENCE

White, L.O.; Reeves, D.S.; Lovering, A.M.; MacGowan, A.P. HPLC assay of cefixime in serum and CSF. *J.Antimicrob.Chemother.*, **1993**, *31*, 450–451

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with buffer, centrifuge, inject a 20 μ L aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with buffer, centrifuge, inject a 20 μ L 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 μ L 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 μ L aliquot. Pleural. Dilute human pleural samples with buffer, centrifuge, inject a 20 μ L aliquot. (Buffer was 66.6 mM K₂HPO₄ adjusted to pH 7.40 with KH₂PO₄.)

HPLC VARIABLES

Column: 200 \times 4 5 μ m Nucleosil C18

Mobile phase: MeOH:buffer 15:85, adjusted to pH 5.2 with phosphoric acid (Buffer was 57.4 mM K₂HPO₄ adjusted to pH 5.2 with phosphoric acid.)

Flow rate: 1

Injection volume: 20-100

Detector: UV 230

CHROMATOGRAM

Retention time: 9

Limit of detection: 100 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: blood, urine

Sample preparation: Serum. 250 μ L Serum + 250 μ L 4 μ g/mL IS in 6% trichloroacetic acid in water, vortex at high speed for 15 s, centrifuge at 30000 g for 2 min, inject a 75 μ L aliquot of the supernatant. Urine. 100 μ L Urine + 2 mL 0.25 μ g/mL IS in 6% trichloroacetic acid in water, vortex at high speed for 15 s, centrifuge at 30000 g for 2 min, inject a 75 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 100 \times 8.5 μ m Nova-Pak C18 RCM-100

Mobile phase: MeCN:water:85% phosphoric acid 17:82.8:0.2 containing 1.36 g/L KH_2PO_4 , pH 2.7 (serum) or MeCN:water:85% phosphoric acid 20:79.8:0.2 containing 1.36 g/L KH_2PO_4 , pH 2.7 (urine)

Flow rate: 2

Injection volume: 75

Detector: UV 280 (serum); UV 313 (urine)

CHROMATOGRAM

Retention time: 3.5 (serum), 4 (urine)

Internal standard: 7-hydroxycoumarin (5.6 (serum), 6.8 (urine))

Limit of quantitation: 50 ng/mL (serum); 1000 ng/mL (urine)

OTHER SUBSTANCES

Noninterfering: acetaminophen, caffeine, salicylic acid, theophylline

KEY WORDS

serum; human; rat; dog; monkey

REFERENCE

Falkowski, A.J.; Look, Z.M.; Noguchi, H.; Silber, B.M. Determination of cefixime in biological samples by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *422*, 145-152

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute serum 1:10 with buffer, dilute urine 1:10-100 with buffer, centrifuge at 9300 g for 4 min, inject 20 μ L of supernatant. (Buffer was 60 mmol K_2HPO_4 adjusted to pH 7.40 with 50 mmol KH_2PO_4 (Soerensen buffer).)

HPLC VARIABLES

Column: 200 \times 4 Nucleosil 5 C18

Mobile phase: MeOH:43 mM K_2HPO_4 15:85 adjusted to pH 5.20 with phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 12

Limit of detection: 100 ng/mL

KEY WORDS

serum

REFERENCE

Knöller, J.; Schönfeld, W.; Bremm, K.D.; König, W. *In vitro* stability of cefixime (FK-027) in serum, urine and buffer. *J.Chromatogr.*, **1987**, *389*, 312–316

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Liu, G.L.; Sha, R.G.; Gao, S.; Shen, Y.X.; Wang, S.X. [Determination of cefixime in human plasma and urine using high performance liquid chromatography column switching technique]. *Yao Hsueh Hsueh Pao*, **1993**, *28*, 216–221

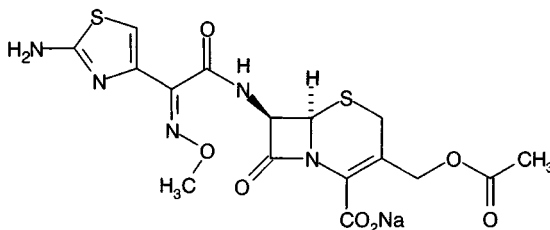
Nahata, M.C. Measurement of cefixime in serum and cerebrospinal fluid by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1991**, *14*, 3755–3759 [serum; CSF; 7-hydroxycoumarin (IS); LOD 30 ng/mL]

Cefotaxime

Molecular formula: C₁₆H₁₇N₅O₇S₂

Molecular weight: 455.5

CAS Registry No.: 63527-52-6 (cefotaxime),
64485-93-4 (cefotaxime sodium)



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: 250 × 4.6 5 μm Ultrasphere ODS

Mobile phase: 8:92 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid

Flow rate: 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 8.4

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, 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: 100 μL Serum + 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:100 mM phosphate 8:92, pH 5.6

Flow rate: 1.2

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Internal standard: cefotaxime

OTHER SUBSTANCES

Extracted: cefaclor

KEY WORDS

serum; mouse; cefotaxime is IS

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

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 μ L Plasma + 1 mL 1 μ g/mL cefaclor in 1% phosphoric acid + 200 μ L MeCN:1% phosphoric acid 1:99, add to the SPE cartridge, wash with 1 mL MeOH:1% phosphoric acid 5:95, wash with 500 μ L 1% phosphoric acid, elute the contents of the SPE cartridge onto the analytical column with the mobile phase.

HPLC VARIABLES

Guard column: 12 \times 4.6 7 μ m Newguard C8

Column: 250 \times 4.6 5 μ m 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: 18.2

Internal standard: cefaclor (14.1)

OTHER SUBSTANCES

Extracted: caffeine, cefpodoxime

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

Sample preparation: 500 μ L Serum + 500 μ L ice-cold 100 μ g/mL cefoperazone 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 μ L of supernatant.

HPLC VARIABLES

Guard column: 10 μ m 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 18:82

Flow rate: 2

Injection volume: 15

Detector: UV 254

CHROMATOGRAM

Internal standard: cefoperazone

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: 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: cefoxitin, cefuroxime, cephalixin, cephaloridine

Noninterfering: alclofenac, aspirin, caffeine, cefadroxil, cefamandole, cefazolin, cefoperazone, cefotiam, cephalothin, diclofenac, ibuprofen, indomethacin, ketoprofen, lonazolac, mefenamic acid, naproxen, phenylbutazone, piroxicam

KEY WORDS

plasma; column-switching; cefotaxime is IS; rat; human

REFERENCE

Lee, Y.J.; Lee, H.S. Simultaneous determination of cefoxitin, cefuroxime, cephalixin and cephaloridine in plasma using HPLC and a column-switching technique. *Chromatographia*, **1990**, *30*, 80–84

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 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 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 \times 4.6 7 μ m 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: 4.1
Internal standard: cefotaxime

OTHER SUBSTANCES

Extracted: cefotiam

KEY WORDS

plasma; cefotaxime is IS

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: 200 μ L Serum + 100 μ L 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 μ L aliquot of the upper aqueous layer.

HPLC VARIABLES

Guard column: 50 mm long CO:PELL ODS
Column: 300 \times 3.9 μ Bondapak 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: 9
Internal standard: cefotaxime
Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: ceftizoxime

Simultaneous: cefamandole, cefazolin, cefoxitin, ceftriaxone, cephalixin, cephaloridine, cephalirin, moxalactam

Noninterfering: amikacin, apalcillin, carbenicillin, cefoperazone, clindamycin, erythromycin, gentamicin, penicillin, piperacillin, ticarcillin, tobramycin, vancomycin

KEY WORDS

serum; cefotaxime is IS; this assay can be used for cefotaxime-see *Antimicrob. Agents Chemother.* 1987; 31; 1177

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:** 300 μ L Plasma + 300 μ L 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 μ L aliquot.

HPLC VARIABLES**Guard column:** 4 \times 4 10 μ m C18**Column:** 300 \times 4 10 μ m μ Bondapak 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:** 6**Internal standard:** 8-chlorotheophylline (4)**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Extracted:** cefoperazone, cefoxitin, cephalixin, cephaloridine

KEY WORDS

plasma

REFERENCESigns, 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 serum with an equal volume of 250 μ g/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 μ L aliquot of the supernatant.

HPLC VARIABLES**Guard column:** RCSS Guard-Pak (Waters)**Column:** 100 \times 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:** 3.0**Internal standard:** 4'-nitroacetanilide (12.4)**Limit of detection:** 3 μ g/mL

OTHER SUBSTANCES**Extracted:** cefamandole, cefazolin, cefoxitin, cephapirin, chloramphenicol**Simultaneous:** acetaminophen, N-acetylprocainamide, cefaclor, cephalixin, 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: 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 μ L serum to SPE cartridge, add 500 μ L PBS to SPE cartridge, wash with 4 mL PBS, elute with 5 mL 1 M NaCl, inject a 100 μ L aliquot of the eluate. (PBS was 8 g NaCl, 1.15 g Na₂HPO₄, 0.2 g KCl, and 0.2 g KH₂PO₄ in 1 L water, pH 7.2.)

HPLC VARIABLES

Column: 300 \times 4 10 μ m octadecylsilane

Mobile phase: MeCN:buffer 13:87 (Buffer was water adjusted to pH 2.8 with acetic acid, about 1.5 mL/L.)

Flow rate: 1.5

Injection volume: 100

Detector: UV 270

CHROMATOGRAM

Retention time: 7.7

Limit of quantitation: 1 μ g/mL

OTHER SUBSTANCES

Extracted: metabolites, 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: cefoxitin

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, urine

Sample preparation: Plasma. 200 μ L Plasma + 20 μ L 0.45 N phosphoric acid + 100 μ L methanol + 20 μ L 270 μ mol/L cephalixin, vortex 15 s, centrifuge for 3 min, remove 100 μ L supernatant, inject 20 μ L. Urine. 10 μ L Urine + 0.5 mL water + 20 μ L 270 μ mol/L cephalixin, vortex 15 s, remove 100 μ L supernatant, inject 20 μ L.

HPLC VARIABLES

Guard column: 100 \times 4.7 Co:Pell ODS

Column: 120 \times 4.7 LiChrosorb RP-18

Mobile phase: MeOH: 10 mM tetrabutylammonium hydrogen sulfate and 20 mM K₃PO₄ and 20 mM KH₂PO₄ 20:80

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 11

Internal standard: cephalixin

Limit of detection: 1 nmol/mL (plasma); 50 nmol/mL (urine)

OTHER SUBSTANCES

Simultaneous: cefotiam

KEY WORDS

plasma

REFERENCE

Lecaillon, J.B.; Rouan, M.C.; Souppart, C.; Febvre, N.; Juge, F. Determination of cefsulodin, cefotiam, cephalixin, cefotaxime, deacetylcefotaxime, cefuroxime and cefroxadin in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *228*, 257–267

SAMPLE

Matrix: bulk, formulations

Sample preparation: Dissolve in water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:water:acetic acid 30:70:0.1

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8.5

Limit of quantitation: 760 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities, cefaclor, cefadroxil, cefamandole, cefamandole nafate, cefazolin, cefoperazone, cefoxitin, ceftizoxime, cephalixin, cephalothin, cephapirin, cephradine

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: Filter (0.45 μ m).

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m 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: 4.6

OTHER SUBSTANCES**Extracted:** cefpirome**Interfering:** carumonam (UV 295), ceftriaxone

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:** formulations**Sample preparation:** Mix an aliquot with an equal volume of 5 mg/mL cefoxitin, dilute with water, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 3.9 5 μ m Resolve (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:** 2.3**Internal standard:** cefoxitin (3.0)

OTHER SUBSTANCES**Simultaneous:** metronidazole

KEY WORDS

stability-indicating; injections; saline

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:** formulations**Sample preparation:** Dilute 1:5 with water, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 3.9 4 μ m μ Bondapak C18**Mobile phase:** MeCN:buffer 7:93 (Buffer was 20 mM KH_2PO_4 and 5 mM triethylamine adjusted to pH 4.8 with NaOH.)**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 270

CHROMATOGRAM**Retention time:** 8.44

OTHER SUBSTANCES**Simultaneous:** desacetylcefotaxime, metronidazole

KEY WORDS

stability-indicating; injections; water

REFERENCE

Rivers, T.E.; McBride, H.A.; Trang, J.M. Stability of cefotaxime sodium and metronidazole in an i.v. admixture at 8°C. *Am.J.Hosp.Pharm.*, **1991**, *48*, 2638–2640

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a 50 µg/mL solution in water.

HPLC VARIABLES

Guard column: 4 × 4 5 µm Lichrospher 100 C18

Column: 250 × 4 5 µm Lichrospher 100 C18

Mobile phase: MeOH:buffer 20:80 (Buffer was 3.5 g KH₂PO₄ and 11.6 g Na₂HPO₄·12H₂O in 1 L water.)

Flow rate: 1

Injection volume: 20

Detector: UV 254

KEY WORDS

comparison with CE

REFERENCE

Fabre, H.; Castaneda Penalvo, G. Capillary electrophoresis as an alternative method for the determination of cefotaxime. *J.Liq.Chromatogr.*, **1995**, *18*, 3877–3887

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 µm Spherisorb ODS II

Mobile phase: Gradient. A was MeOH:buffer 4:20. B was MeOH:buffer 7:20. A:B 100:0 for 8 min, to 0:100 over 21 min, maintain at 0:100 for 4 min. Re-equilibrate at initial conditions for 2 min. (Buffer was KH₂PO₄/Na₂HPO₄.)

Column temperature: 25

Flow rate: 1

Detector: UV 235

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Wetterich, U.; Mutschler, E. Quality of cefotaxime sodium preparations. *Arzneimittelforschung*, **1995**, *45*, 74–80

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 18:82

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.6

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: Dissolve in 100 mM pH 10.5 carbonate buffer at a concentration of 0.113 mM, inject 50 μ L aliquots.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Spherisorb ODS-2

Mobile phase: MeCN:10 mM ammonium acetate 6:94, pH 6.50

Flow rate: 1.1

Injection volume: 50

Detector: UV 235

CHROMATOGRAM

Retention time: 9.28

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Vilanova, B.; Muñoz, F.; Donoso, J.; Frau, J.; Garcia Blanco, F. Alkaline hydrolysis of cefotaxime. A HPLC and 1 H NMR study. *J.Pharm.Sci.*, **1994**, 83, 322–327

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 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:10 mM ammonium acetate 10:90

Flow rate: 1.5

Injection volume: 10-20

Detector: UV 240

OTHER SUBSTANCES

Also analyzed: cefuroxime, cephacetrile

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: 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: 9.5

OTHER SUBSTANCES

Simultaneous: 7-aminocephalosporanic acid, cefadroxil, cefazolin, cephalixin, 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

ANNOTATED BIBLIOGRAPHY

Changqin, H.; Shaohong, S.; Kaimin, W. The chromatographic behavior of cephalosporins in gel filtration chromatography, a novel method to separate high molecular weight impurities. *J.Pharm. Biomed.Anal.*, **1994**, *12*, 533–541 [also cefadroxil, cefamandole, cefmenoxime, cefoperazone, ceftazidime, ceftriaxone, cephalixin, cephaloridine, cephalothin, cephradine]

Fabre, H.; Fell, A.F. Comparison of techniques for peak purity testing of cephalosporins. *J.Liq.Chromatogr.*, **1992**, *15*, 3031–3043 [also theophylline]

Haginaka, J.; Yasuda, N.; Wakai, J.; Matsunaga, H.; Yasuda, H.; Kimura, Y. Internal-surface reversed-phase silica support for direct injection determination of drugs in biological fluids by liquid chromatography. *Anal.Chem.*, **1989**, *61*, 2445–2448 [direct injection; ISRP; serum; extracted cefamandole, cefmenoxime]

Paap, C.M.; Nahata, M.C. A novel micromethod for the simultaneous analysis of cefotaxime and desacetylcefotaxime from plasma using ion pair high performance liquid chromatography. *J.Liq.Chromatogr.*, **1989**, *12*, 2385–2395 [plasma; cefoxitin (IS); also acetaminophen, caffeine, cefazolin, methicillin, theophylline, vancomycin; non-interfering ampicillin, gentamicin, ibuprofen, phenobarbital]

Hakim, L.; Bourne, D.W.; Triggs, E.J. High-performance liquid chromatographic assay of cefotaxime, desacetylcefotaxime and ceftriaxone in rat plasma. *J.Chromatogr.*, **1988**, *424*, 111–117

Hary, L.; Andrejak, M. [Analysis of serum cefotaxime and desacetylcefotaxime by high performance liquid ion exchange chromatography]. *J.Chromatogr.*, **1987**, *419*, 396–400

Yost, R.L.; Derendorf, H. Rapid chromatographic determination of cefotaxime and its metabolite in biological fluids. *J.Chromatogr.*, **1985**, *341*, 131–138

Bawdon, R.E.; Novick, W.J.; Hemsell, D.L.; Welch, W.D. High-pressure liquid chromatographic assay of cefotaxime and desacetylcefotaxime in human yodometry. *J.Liq.Chromatogr.*, **1984**, *7*, 2483–2491 [SPE]

Demotes-Mainard, F.; Vinçon, G.; Bouchet, J.L.; Jarry, C.; Albin, H. [Assay of cefotaxime and desacetylcefotaxime in plasma and urine by high performance liquid chromatography]. *Ann Biol.Clin.(Paris)*, **1984**, *42*, 301–305

LeBel, M.; Ericson, J.F.; Pitkin, D.H. Improved high-performance liquid chromatographic (HPLC) assay method for ceftizoxime. *J.Liq.Chromatogr.*, **1984**, *7*, 961–968 [simultaneous ceftizoxime; cefotaxime is IS]

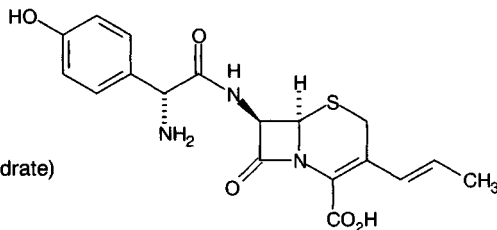
Nygaard, G.; Wahba Kahlil, S.K. An isocratic HPLC method for the determination of cephalosporins in plasma. *J.Liq.Chromatogr.*, **1984**, *7*, 1461–1475 [plasma; cephapirin (IS); column temp 45; extracted cefamandole, cefazolin, cefonicid, cefoperazone, cefoxitin, cephalothin]

Cefprozil

Molecular formula: C₁₈H₁₉N₃O₅S

Molecular weight: 389.4

CAS Registry No.: 92665-29-7, 121123-17-9 (monohydrate)



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 150 μ L 300 μ g/mL cephalixin + 20 μ L 5% trichloroacetic acid + 100 μ L MeCN, vortex, centrifuge at 13000 g for 3 min, inject a 40 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 37 μ m Corasil C18

Column: 100 \times 9.4 Partisil-5-CCS-C8RAC C8

Mobile phase: MeCN:3 mM pH 3.8 sodium acetate buffer 13:87

Flow rate: 0.9

Injection volume: 40

Detector: UV 280

CHROMATOGRAM

Retention time: 10.2

Internal standard: cephalixin (12.4)

Limit of quantitation: 500 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Barbhaiya, R.H.; Shukla, U.A.; Gleason, C.R.; Shyu, W.C.; Wilber, R.B.; Martin, R.R.; Pittman, K.A. Phase I study of multiple-dose cefprozil and comparison with cefaclor. *Antimicrob. Agents Chemother.*, **1990**, *34*, 1198–1203

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 100 μ L 1 mg/mL cephalixin + 150 μ L 10% trichloroacetic acid + 500 μ L MeCN, mix, add 1.5 mL dichloromethane, vortex, centrifuge, inject a 60 μ L aliquot of the aqueous supernatant. Urine. 5 mL Urine + 5 mL 20 mM pH 3.8 sodium acetate buffer, mix. 500 μ L Buffered urine + 100 μ L 1.5 mg/mL cephalixin + 150 μ L 5% trichloroacetic acid, vortex, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 45 \times 4 37-50 μ m C18/Corasil

Column: Zorbax C8 (plasma) or Partisil 5 ODS-3 RAC C18 (urine)

Mobile phase: MeCN:glacial acetic acid:water 17:2:81 (plasma) or MeCN:MeOH:THF:trichloroacetic acid:glacial acetic acid:sodium acetate trihydrate:sodium dodecyl sulfate:water 25:3:0.925:0.075:0.25:0.077:0.134:70.75 (v/v/v/w/v/w/w/v) (urine)

Flow rate: 1.5 (plasma) or 2 (urine)

Injection volume: 10-60

Detector: UV 280

CHROMATOGRAM

Retention time: 14 (cis, plasma), 15 (cis, urine), 18 (trans, urine), 20 (trans, plasma)

Internal standard: cephalixin (17 (plasma), 23 (urine))

Limit of quantitation: 100 ng/mL (plasma); 5000 ng/mL (urine)

KEY WORDS

plasma; human; rat; pharmacokinetics

REFERENCE

Shyu, W.C.; Shukla, U.A.; Shah, V.R.; Papp, E.A.; Barbhaiya, R.H. Simultaneous high-performance liquid chromatographic analysis of cefprozil diastereomers in a pharmacokinetic study. *Pharm.Res.*, 1991, 8, 992-996

SAMPLE

Matrix: urine

Sample preparation: Mix urine with an equal volume of 20 mM pH 3.57 sodium acetate buffer. 500 μ L Buffered urine + 100 μ L 1.5 μ g/mL cephalixin + 150 μ L 5% trichloroacetic acid, vortex for 30 s, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 37 μ m Corasil C18

Column: 100 \times 9.4 Partisil-5-ODS-3RAC C18

Mobile phase: MeCN:MeOH:THF:buffer 25:3:0.925:71.075 (Prepare buffer by dissolving 1.54 g sodium acetate trihydrate and 2.67 g sodium dodecyl sulfate in 1 L water and adding 5 mL glacial acetic acid, 30 mL 5% trichloroacetic acid, 500 mL MeCN, 60 mL MeOH, and 18.5 mL THF, make up to 2 L with water.)

Flow rate: 2

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 12

Internal standard: cephalixin (17)

Limit of quantitation: 5 μ g/mL

KEY WORDS

pharmacokinetics

REFERENCE

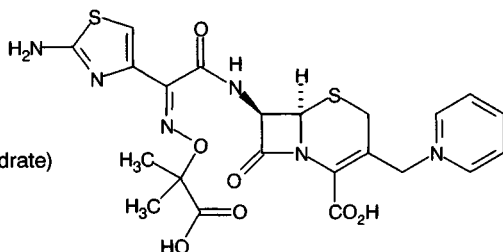
Barbhaiya, R.H.; Shukla, U.A.; Gleason, C.R.; Shyu, W.C.; Wilber, R.B.; Martin, R.R.; Pittman, K.A. Phase I study of multiple-dose cefprozil and comparison with cefaclor. *Antimicrob.Agents Chemother.*, 1990, 34, 1198-1203

Ceftazidime

Molecular formula: C₂₂H₂₂N₆O₇S₂

Molecular weight: 546.6

CAS Registry No.: 72558-82-8, 78439-06-2 (pentahydrate)



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: MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid 9:91

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 6.3

Limit of detection: LOD 200 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, ceftiofur, ceftiofur sodium, ceftiofur sodium, ceftiofur sodium, cefotaxime, 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: 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 20 μL aliquot of the aqueous layer. Blister fluid. Inject directly.

HPLC VARIABLES

Column: μBondapak C18

Mobile phase: MeCN:100 mM phosphate buffer 2.5:97.5

Flow rate: 2

Injection volume: 20

Detector: UV 229

CHROMATOGRAM**Retention time:** 14

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: Dilute serum with 3 volumes 10 mM NaH₂PO₄, inject a 50 µL aliquot onto column A and elute to waste with mobile phase A, after 0.9 min elute the contents of column A onto column B with mobile phase A, after 2.6 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 4 40 µm C8 (Backflush each day with MeCN:water 50:50 for 20 min.); B 150 × 4 5 µm HP ODS (Hewlett-Packard)

Mobile phase: A MeCN:10 mM NaH₂PO₄ 4:96, pH 5.0; B MeCN:10 mM NaH₂PO₄ 2:92, pH 5.0 (sic, 8:92 ?)

Flow rate: 1**Injection volume:** 50**Detector:** UV 258

CHROMATOGRAM**Retention time:** 8.9**Limit of detection:** 500 ng/mL

KEY WORDS

serum; column-switching; pharmacokinetics

REFERENCE

Bompadre, S.; Ferrante, L.; Alò, F.P.; Leone, L. On-line solid-phase extraction of ceftazidime in serum and determination by high-performance liquid chromatography. *J. Chromatogr. B*, **1995**, *669*, 265–269

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**Limit of quantitation:** 500 ng/mL

KEY WORDS

pharmacokinetics; 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

Sample preparation: 50 μ L Serum + 50 μ L 50 μ g/mL cephaloridine in 6% perchloric acid, mix, centrifuge at 1500 g for 5 min, inject a 25 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: present but not specified

Column: 100 \times 8 Resolve C18 Radial Pak glass column (Waters)

Mobile phase: MeCN:MeOH:20 mM pH 3.6 sodium acetate buffer 4.8:13.5:81.7

Flow rate: 2

Injection volume: 25

Detector: UV 254; UV 265

CHROMATOGRAM

Internal standard: cephaloridine

Limit of detection: 500 ng/mL

KEY WORDS

pharmacokinetics; serum

REFERENCE

van den Anker, J.N.; Schoemaker, R.C.; Hop, W.C.J.; van der Heijden, B.J.; Weber, A.; Sauer, P.J.J.; Neijens, H.J.; de Groot, R. Ceftazidime pharmacokinetics in preterm infants: Effects of renal function and gestational age. *Clin. Pharmacol. Ther.*, **1995**, *58*, 650–659

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 500 μ L MeCN, vortex for 10 s, centrifuge at 3000 g for 10 min. Remove 800 μ L of the supernatant and add it to 5 mL dichloromethane, vortex for 10 s, centrifuge, inject a 50 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 20 \times 4.6 10 μ m Spherisorb C8

Column: 250 \times 4.6 5 μ m 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: 19.8

Internal standard: ceftazidime

OTHER SUBSTANCES

Extracted: ceftibuten

Noninterfering: acyclovir, amikacin, norfloxacin, ofloxacin, pefloxacin, tobramycin

KEY WORDS

plasma; column-switching; ceftazidime is IS

REFERENCE

Kinowski, J.M.; Bressolle, F.; Fabre, D.; Goncalves, F.; Rouzier-Panis, R.; Galtier, M. High-performance liquid chromatographic determination of cefibuten and its metabolite in biological fluids: Applications in pharmacokinetic studies. *J.Pharm.Sci.*, **1994**, *83*, 736-741

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 500 μ L MeCN:EtOH:water (40:40:20), vortex for 10-15 s, centrifuge through a Centricon-10 filter unit with a 10000 dalton cut-off (Amicon) at 4000 g for 30 min, inject a 10-60 μ L aliquot of the colorless ultrafiltrate.

HPLC VARIABLES

Column: 150 \times 4.6 3 μ m Ultremex phenyl (Phenomenex)

Mobile phase: MeCN:water 25:75 containing 5 mM sodium dodecanesulfonate and 0.1% phosphoric acid

Column temperature: 40

Flow rate: 0.8

Injection volume: 10-60

Detector: UV 259

CHROMATOGRAM

Retention time: 7-8

Limit of detection: 50 ng/mL

KEY WORDS

serum; dolphin; ultrafiltrate

REFERENCE

Tyczkowska, K.L.; Seay, S.S.; Stoskopf, M.K.; Aucoin, D.P. Determination of ceftazidime in dolphin serum by liquid chromatography with ultraviolet-visible detection and confirmation by thermospray liquid chromatography-mass spectrometry. *J.Chromatogr.*, **1992**, *576*, 305-313

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 500 μ L ice-cold 50 μ g/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 μ L of supernatant.

HPLC VARIABLES

Guard column: 10 μ m C18 Guard-PAK

Column: 300 \times 3.9 10 μ m μ Bondapak 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: ceftizoxime

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 μ L Plasma + 500 μ L MeCN, mix vigorously on a Whirlmixer for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μ L of the supernatant and add it to 3 mL dichloromethane, centrifuge at 1200 g for 5 min, inject a 20 μ L aliquot of the upper aqueous layer.

HPLC VARIABLES**Column:** 100 \times 3.5 μ m 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:** 500 ng/mL

OTHER SUBSTANCES**Also analyzed:** cefepime, ceftriaxone

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, CSF**Sample preparation:** Deproteinize serum or CSF with MeCN, centrifuge, add the supernatant to dichloromethane, inject a 100 μ L 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 pentane-sulfonic 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: blood, dialysate

Sample preparation: Plasma. 0.5 mL Plasma + 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 (J.Chromatogr. 1987, 413, 109). Dialysate. Inject a 30 µL aliquot directly.

HPLC VARIABLES

Mobile phase: MeCN:100 mM (sic) pH 3.5 acetate buffer:40 mM tetradecyltrimethylammonium bromide 20:78:2

Flow rate: 1

Injection volume: 30

Detector: UV 270

CHROMATOGRAM

Limit of detection: 50 ng/mL

KEY WORDS

rat; plasma; pharmacokinetics

REFERENCE

Granero, L.; Santiago, M.; Cano, J.; Machado, A.; Peris, J.-E. Analysis of ceftriaxone and ceftazidime distribution in cerebrospinal fluid of and cerebral extracellular space in awake rats by in vivo microdialysis. *Antimicrob. Agents Chemother.*, **1995**, *39*, 2728–2731

SAMPLE

Matrix: blood, urine

Sample preparation: Add aminophylline, precipitate proteins with 0.8 M perchloric acid, inject an aliquot.

HPLC VARIABLES

Column: µBondapak C18

Mobile phase: MeOH:90 mM sodium acetate 14:86, pH adjusted to 4.2 with acetic acid

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 7.2

Internal standard: aminophylline

Limit of detection: 150 ng/mL

OTHER SUBSTANCES

Also analyzed: ceftiofime

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 ceftiprome, ceftazidime, ceftriaxone, imipenem, and ciprofloxacin. *Antimicrob. Agents Chemother.*, **1992**, *36*, 2085–2092

SAMPLE

Matrix: cecal contents

Sample preparation: Dilute cecal contents in 2 mL phosphate-buffered saline, centrifuge at 1500 g for 10 min. 500 μ L Sample + 500 μ L MeCN, vortex for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μ L of the supernatant and add it to 3 mL dichloromethane, mix for 30 s, centrifuge at 1200 g for 5 min, inject a 20 μ L aliquot of the upper aqueous phase.

HPLC VARIABLES

Column: 100 \times 3 5 μ m 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: cefoperazone, 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: formulations

Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m cyano

Mobile phase: MeCN:100 mM NaH₂PO₄ 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:** formulations**Sample preparation:** Dilute 100 μL eye drops to 25 mL with water, inject a 10 μL aliquot.

HPLC VARIABLES**Guard column:** 10 \times 4.6 5 μm Spherisorb hexyl**Column:** 100 \times 4.6 5 μm Spherisorb hexyl**Mobile phase:** MeCN:50 mM pH 7.0 ammonium acetate 7:93**Flow rate:** 2**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 1.3

OTHER SUBSTANCES**Simultaneous:** degradation products, pyridine

KEY WORDS

stability-indicating; eye drops

REFERENCEBarnes, A.R. Determination of cefotaxime and pyridine by HPLC: Application to a viscous eye drop formulation. *J.Liq.Chromatogr.*, **1995**, *18*, 3117–3128

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute 1000-fold, mix a 200 μL aliquot with 200 μL 100 $\mu\text{g/mL}$ hydrochlorothiazide, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** Microsorb MV C-18**Mobile phase:** MeCN:water:acetic acid 6:93:1, adjusted to pH 4.0 with 6 M NaOH**Flow rate:** 2**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Internal standard:** hydrochlorothiazide

KEY WORDS

injections; saline; stability-indicating

REFERENCEBednar, D.A.; Klutman, N.E.; Henry, D.W.; Fox, J.L.; Strayer, A.H. Stability of cefotaxime (with arginine) in an elastomeric infusion device. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1912–1914

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute 50-fold with water, inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 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: 1.7

OTHER SUBSTANCES

Simultaneous: ceftizoxime, 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: 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 \times 4.6 5 μm Adsorbosphere C18

Mobile phase: MeCN:50 mM pH 7 phosphate buffer 7.5:92.5

Flow rate: 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 6.8

KEY WORDS

stability-indicating; injections; 5% dextrose

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 with water, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 5 μm Nova Pak C18

Mobile phase: MeOH:5 mM pH 7.5 phosphate buffer 10:90

Flow rate: 0.6

Detector: UV 254

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

Simultaneous: degradation products

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 (100 µg/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: 4.6

Internal standard: theophylline (3.3)

OTHER SUBSTANCES

Simultaneous: cefazolin

KEY WORDS

stability-indicating; 5% dextrose; injections

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: solutions

Sample preparation: Dilute with an equal volume of water or buffer, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb C6

Mobile phase: MeCN:buffer 4:96 (Buffer was 100 mM acetic acid containing 25 mM sodium acetate.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8.9

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

stability-indicating

REFERENCE

Zhou, M.; Notari, R.E. Influence of pH, temperature, and buffers on the kinetics of ceftazidime degradation in aqueous solution. *J.Pharm.Sci.*, **1995**, *84*, 534–538

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 12:88

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.4

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: Dilute 1:100 with water.

HPLC VARIABLES

Column: Novapak C18 (model no. PN86344)

Mobile phase: MeCN:glacial acetic acid:water 6:1:93, adjusted to a pH of 4.0

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Interfering: cefuroxime

KEY WORDS

water; stability-indicating

REFERENCE

Stiles, M.L.; Allen, L.V., Jr.; Fox, J.L. Stability of ceftazidime (with arginine) and of cefuroxime sodium in infusion-pump reservoirs. *Am.J.Hosp.Pharm.*, **1992**, *49*, 2761–2764

SAMPLE

Matrix: urine

Sample preparation: Dilute urine three-fold with 200 mM pH 4.5 sodium acetate buffer, vortex for 30 s, inject a 10 µL aliquot.

HPLC VARIABLES

Guard column: 23 × 4 37-50 μm Corasil C18

Column: 100 × 9.4 Partisil 5 ODS-3RAC C18

Mobile phase: 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

Flow rate: 2.8

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 10

Internal standard: cefotaxime

OTHER SUBSTANCES

Extracted: cefepime

KEY WORDS

rat; cefotaxime is IS

REFERENCE

Barbhaiya, R.H.; Fogue, S.T.; Shyu, W.C.; Papp, E.A.; Pittman, K.A. High-pressure liquid chromatographic analysis of BMV-28142 in plasma and urine. *Antimicrob. Agents Chemother.*, **1987**, *31*, 55–59

ANNOTATED BIBLIOGRAPHY

Changqin, H.; Shaohong, S.; Kaimin, W. The chromatographic behavior of cephalosporins in gel filtration chromatography, a novel method to separate high molecular weight impurities. *J. Pharm. Biomed. Anal.*, **1994**, *12*, 533–541 [also cefadroxil, cefamandole, cefmenoxime, cefoperazone, cefotaxime, ceftriaxone, cephalixin, cephaloridine, cephalothin, cephradine]

Fauzi, M.A.; Dine, T.; Luyckx, M.; Gressier, B.; Brunet, C.; Goudaliez, F.; Mallevais, M.L.; Cazin, M.; Cazin, J.C. Stability and compatibility studies of cefaloridine, cefuroxime and cefotaxime with PVC infusion bags. *Pharmazie*, **1994**, *49*, 425–427 [also cefaloridine, cefuroxime; formulations; saline; 5% dextrose]

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: Application in pharmacokinetic studies. *J. Pharm. Sci.*, **1994**, *83*, 736–741 [plasma; extracted ceftibuten; ceftazidime is IS]

Vinks, A.A.T.M.M.; Touw, D.J.; Heijerman, H.G.M.; Danhof, M.; de Leede, G.P.J.; Bakker, W. Pharmacokinetics of ceftazidime in adult cystic fibrosis patients during continuous infusion and ambulatory treatment at home. *Ther. Drug Monit.*, **1994**, *16*, 341–348 [pharmacokinetics; serum; urine; sputum; LOD 500 ng/mL; 8-chlorotheophylline (IS)]

Nahata, M.C.; Morosco, R.S. Measurement of ceftazidime arginine in aqueous solution by HPLC. *J. Liq. Chromatogr.*, **1992**, *15*, 1507–1511

Fasching, C.E.; Peterson, L.R.; Gerding, D.N. High pressure liquid chromatographic analysis for the quantitation of BMV-28142 and ceftazidime in human and rabbit serum. *J. Liq. Chromatogr.*, **1986**, *9*, 1803–1814 [human; rabbit; serum; extracted BMV-28142; LOD 1.5 μg/mL]

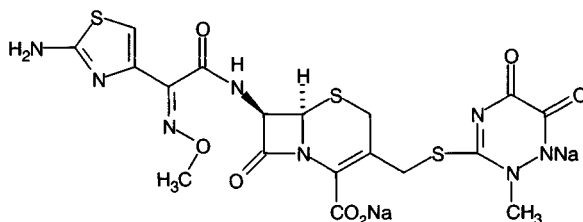
Hwang, P.T.R.; Drexler, P.G.; Meyer, M.C. High-performance liquid chromatographic determination of ceftazidime in serum, urine, CSF, and peritoneal dialysis fluid. *J. Liq. Chromatogr.*, **1984**, *7*, 979–987 [serum; urine; CSF; peritoneal dialysis fluid; hydrochlorothiazide (IS); also amikacin, ampicillin, caffeine, chloramphenicol]

Ceftriaxone

Molecular formula: C₁₈H₁₈N₈O₇S₃

Molecular weight: 554.6

CAS Registry No.: 73384-59-5 (ceftriaxone),
104376-79-6 (ceftriaxone sodium)



SAMPLE

Matrix: bile

Sample preparation: 100 μ L Bile + 200 μ L water, vortex for 20 s, add 3 mL 45.2 μ g/mL o-phthalic acid in mobile phase, vortex, centrifuge, inject a 20-50 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 3.2 5 μ m LiChrosorb RP-18

Mobile phase: MeCN:pH 7.0 Titrisol phosphate buffer (Merck):water 440:60:500, containing 3.5 g/L tetraoctylammonium bromide

Flow rate: 1.5

Injection volume: 20-50

Detector: UV 274

CHROMATOGRAM

Retention time: 7.5

Internal standard: o-phthalic acid (10.1)

Limit of detection: 5 μ g/mL

KEY WORDS

dog; human

REFERENCE

Trautmann, K.H.; Haefelfinger, P. Determination of the cephalosporin Ro 13-9904 in plasma, urine, and bile by means of ion-pair reversed phase chromatography. *J.High Res.Chromatogr.*, **1981**, *4*, 54-59

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: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:10 mM phosphate buffered saline containing 11 mM hexadecyltrimethylammonium bromide 50:50, adjusted to pH 8 with glacial acetic acid

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 17.5

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, 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: 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

Sample preparation: 500 μL Serum + 500 μL ice-cold 100 μg/mL cefoperazone 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 35:65

Flow rate: 1.2

Injection volume: 15

Detector: UV 254

CHROMATOGRAM

Internal standard: cefoperazone

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 μ L Plasma + 500 μ L MeCN, mix vigorously on a Whirlmixer for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μ L of the supernatant and add it to 3 mL dichloromethane, centrifuge at 1200 g for 5 min, inject a 20 μ L aliquot of the upper aqueous layer.

HPLC VARIABLES**Column:** 100 \times 3.5 μ m Hypersil ODS**Mobile phase:** MeCN:5 mM pH 5.5 acetate buffer 0.7:97.3**Flow rate:** 1**Injection volume:** 20**Detector:** UV 274

CHROMATOGRAM**Limit of detection:** 400 ng/mL

OTHER SUBSTANCES**Also analyzed:** cefepime, cefoperazone, ceftazidime

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

Sample preparation: 100 μ L Plasma + 300 μ L water, vortex for 20 s, add 2 mL 25 μ g/mL probenecid in EtOH, shake 20-30 times by hand, rotate at 20 rpm for 5 min, centrifuge at 1000 g for 2 min, inject a 60 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 3.2 μ m LiChrosorb RP-18**Mobile phase:** MeCN:pH 7.0 Titrisol phosphate buffer (Merck):water 500:50:450, containing 4 g/L hexadecyltrimethylammonium bromide**Flow rate:** 1.5**Injection volume:** 60**Detector:** UV 274

CHROMATOGRAM**Retention time:** 3.8**Internal standard:** probenecid (6.7)**Limit of detection:** 500 ng/mL

KEY WORDS

plasma; dog; human

REFERENCE

Trautmann, K.H.; Haefelfinger, P. Determination of the cephalosporin Ro 13-9904 in plasma, urine, and bile by means of ion-pair reversed phase chromatography. *J.High Res.Chromatogr.*, **1981**, *4*, 54-59

SAMPLE**Matrix:** blood, CSF, urine**Sample preparation:** Dilute urine 1:10 with normal saline. 50 μ L Serum, CSF, or diluted urine + 50 μ L 200 μ g/mL moxalactam in MeCN, vortex for 30 s, centrifuge at 13000 g for 3 min, inject a 10 μ L aliquot.

HPLC VARIABLES**Guard column:** 30 \times 4 18 μ m spherical silica gel**Column:** 300 \times 4 μ Bondapak C18**Mobile phase:** MeCN:10 mM pH 9.0 potassium phosphate buffer containing 10 mM hexadecyltrimethylammonium bromide 46:54**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV 274

CHROMATOGRAM**Retention time:** 4**Internal standard:** moxalactam (7)**Limit of detection:** 1 μ g/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, ampicillin, carbamazepine, cefamandole, cefazolin, cefoperazone, cefotaxime, cefoxitin, ceftazidime, ceftizoxime, cephalixin, cephalothin, cephapirin, chloramphenicol, ciprofloxacin, clonazepam, cyclosporine, desipramine, digoxin, disopyramide, ethosuximide, gentamicin, haloperidol, imipramine, kanamycin, lidocaine, mezlocillin, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, salicylic acid, streptomycin, sulfamethoxazole, theophylline, thiamphenicol, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum

REFERENCE

Granich, G.G.; Krogstad, D.J. Ion pair high-performance liquid chromatographic assay for ceftriaxone. *Antimicrob.Agents Chemother.*, **1987**, *31*, 385-388

SAMPLE**Matrix:** blood, dialysate**Sample preparation:** Plasma. 0.5 mL Plasma + 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 (*J.Chromatogr.* 1987, 413, 109). Dialysate. Inject a 30 μ L aliquot directly.

HPLC VARIABLES**Mobile phase:** MeCN:MeOH:50 mM pH 6.5 phosphate buffer:40 mM tetradecyltrimethylammonium bromide 30:10:45:15

Flow rate: 1
Injection volume: 30
Detector: UV 270

CHROMATOGRAM

Limit of detection: 50 ng/mL

KEY WORDS

rat; plasma; pharmacokinetics

REFERENCE

Granero, L.; Santiago, M.; Cano, J.; Machado, A.; Peris, J.-E. Analysis of ceftriaxone and ceftazidime distribution in cerebrospinal fluid of and cerebral extracellular space in awake rats by in vivo microdialysis. *Antimicrob. Agents Chemother.*, **1995**, *39*, 2728–2731

SAMPLE

Matrix: blood, urine

Sample preparation: Precipitate proteins with MeCN containing cefotetan.

HPLC VARIABLES

Column: μ Bondapak 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

Retention time: 7.4

Internal standard: cefotetan

Limit of detection: 600 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: blood, urine

Sample preparation: 500 (?) μ L Plasma or urine + 1500 (?) μ L MeCN, vortex, centrifuge, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: Brownlee 10 μ m RP-8

Mobile phase: MeCN:MeOH:20 mM pH 5 phosphate buffer 5:25:70

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Also analyzed: cefaronide, ceftriaxone

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:** cecal contents**Sample preparation:** Weigh contents of cecum, dilute with 2 mL PBS, centrifuge at 1500 g for 10 min. Add a 500 μ L aliquot of supernatant to 500 μ L MeCN, mix on a whirlmixer for 30 s, centrifuge at 1200 g for 5 min. Remove 400 μ L of the supernatant and add it to 3 mL dichloromethane, mix for 30 s, centrifuge at 1200 g for 5 min, inject a 20 μ L aliquot of the upper aqueous phase.

HPLC VARIABLES**Column:** 100 \times 3.5 μ m Hypersil ODS**Mobile phase:** MeCN:5 mM pH 5.5 acetate buffer 0.7:99.3**Flow rate:** 1**Injection volume:** 20**Detector:** UV 274

CHROMATOGRAM**Limit of quantitation:** 500 ng/mL

OTHER SUBSTANCES**Also analyzed:** cefepime, cefoperazone, ceftazidime

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:** formulations**Sample preparation:** Dilute 50-fold with water, inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 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:** 2.1

OTHER SUBSTANCES**Simultaneous:** ceftazidime, ceftizoxime, 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: formulations

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 10 μ m Dynamax C18

Column: 250 \times 4.6 10 μ m Dynamax C18

Mobile phase: MeCN:6.5 mM tetraheptylammonium bromide:100 mM pH 7.0 phosphate buffer:100 mM pH 5.0 citrate buffer 40:55.2:4.4:0.4

Flow rate: 1

Injection volume: 20

Detector: UV 271

CHROMATOGRAM

Retention time: 14.9

OTHER SUBSTANCES

Simultaneous: degradation products, theophylline

KEY WORDS

injections; stability-indicating; saline; use low actinic glassware; 5% dextrose

REFERENCE

Parrish, M.A.; Bailey, L.C.; Medwick, T. Stability of ceftriaxone sodium and aminophylline or theophylline in intravenous mixtures. *Am.J.Hosp.Pharm.*, **1994**, *51*, 92–94

SAMPLE

Matrix: urine

Sample preparation: 100 μ L Urine + 200 μ L water, vortex for 20 s, add 3 mL 9 μ g/mL 4-nitrobenzoic acid in mobile phase, vortex, centrifuge, inject a 20-50 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 3.2 5 μ m LiChrosorb RP-18

Mobile phase: MeCN:buffer:water 200:38:762, containing 3.8 g/L tetrapentylammonium bromide (Buffer was 500 mL of 0.5 M Na_2HPO_4 adjusted to pH 7.8 with about 50 mL 0.5 M NaH_2PO_4 .)

Flow rate: 1.5

Injection volume: 20-50

Detector: UV 300

CHROMATOGRAM

Retention time: 6.4

Internal standard: 4-nitrobenzoic acid (20.4)

Limit of detection: 5 μ g/mL

KEY WORDS

dog; human

REFERENCE

Trautmann, K.H.; Haefelfinger, P. Determination of the cephalosporin Ro 13-9904 in plasma, urine, and bile by means of ion-pair reversed phase chromatography. *J.High Res.Chromatogr.*, **1981**, *4*, 54–59

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- Bailey, L.C.; Tang, K.T.; Medwick, T. Stability of ceftriaxone sodium in infusion-pump syringes. *Am.J.Hosp.Pharm.*, **1993**, *50*, 2092–2094
- 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
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- Mohler, J.; Meulemans, A.; Vulpillat, M. High-performance liquid chromatographic determination of the binding of ceftriaxone to human serum albumin solution and albumin from diluted human serum. *J.Chromatogr.*, **1990**, *528*, 415–423
- Jungbluth, G.L.; Jusko, W.J. Ion-paired reversed-phase high-performance liquid chromatography assay for determination of ceftriaxone in human plasma and urine. *J.Pharm.Sci.*, **1989**, *78*, 968–970
- Hakim, L.; Bourne, D.W.; Triggs, E.J. High-performance liquid chromatographic assay of cefotaxime, desacetylcefotaxime and ceftriaxone in rat plasma. *J.Chromatogr.*, **1988**, *424*, 111–117
- Marini, D.; Balestrieri, F. Determination of ceftriaxone by HPLC. *Farmaco.[Prat.]*, **1986**, *41*, 172–176
- Bawdon, R.E.; Hemsell, D.L.; Hemsell, P.G. Serum and pelvic tissue concentrations of ceftriaxone and ceftazolin at hysterectomy. *J.Liq.Chromatogr.*, **1984**, *7*, 2011–2020
- Bowman, D.B.; Aravind, M.K.; Miceli, J.N.; Kauffman, R.E. Reversed-phase high-performance liquid chromatographic method to determine ceftriaxone in biological fluids. *J.Chromatogr.*, **1984**, *309*, 209–213
- Ascalone, V.; Dal, B. Determination of ceftriaxone, a novel cephalosporin, in plasma, urine and saliva by high-performance liquid chromatography on an NH₂ bonded-phase column. *J.Chromatogr.*, **1983**, *273*, 357–366

Cefuroxime

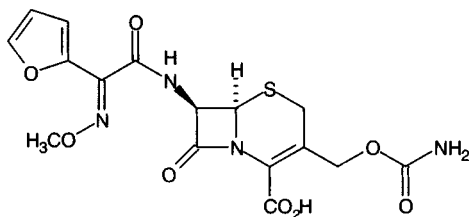
Molecular formula: C₁₆H₁₆N₄O₈S

Molecular weight: 424.4

CAS Registry No.: 55268-75-2, 56238-63-2 (Na salt),

64544-07-6 (cefuroxime axetil), 100680-33-9

(cefuroxime pivoxetil)



SAMPLE

Matrix: blood

Sample preparation: Deproteinize with 12.5% trichloroacetic acid.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Beckman Ultrasphere ODS

Mobile phase: MeCN:50 mM phosphate buffer 10:90

Flow rate: 1.5

Detector: UV 280

CHROMATOGRAM

Retention time: 6.3

Internal standard: cephaloridine

Limit of quantitation: 500 ng/mL

KEY WORDS

serum

REFERENCE

Donn, K.H.; James, N.C.; Powell, J.R. Bioavailability of cefuroxime axetil formulations. *J.Pharm.Sci.*, 1994, 83, 842-844

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 400 μL A, vortex, centrifuge, inject 10 μL supernatant. (A was 1 mg/mL cephalixin in water:20% perchloric acid:water 3:2:7.)

HPLC VARIABLES

Guard column: Used but not specified

Column: 100 × 3 5 μm ChromSpher C18 in a glass column

Mobile phase: MeOH:Sorensen buffer (67 mM KH₂PO₄) 15:85

Flow rate: 0.4

Injection volume: 10

Detector: UV 275

CHROMATOGRAM

Retention time: 7.7

Internal standard: cephalixin

Limit of quantitation: 700 ng/mL

OTHER SUBSTANCES

Noninterfering: diazepam, etomidate, fentanyl, heparin, pancuronium, papaverine, polygeline, procaine, protamine, sodium nitroprusside

KEY WORDS

serum

REFERENCE

Koot, M.J.; IJdenberg, F.N.; Stuurman, R.M.; Poell, J.; Bras, L.J.; Langemeijer, J.J.; Lie-A-Huen, L. High pressure liquid chromatographic analysis of the serum concentration of cefuroxime after an intravenous bolus injection of cefuroxime in patients with a coronary artery bypass grafting. *Pharm.Weekbl.[Sci]*, **1992**, *14*, 360–364

SAMPLE

Matrix: blood

Sample preparation: Mix 100 μL plasma + 300 μL 5 $\mu\text{g}/\text{mL}$ 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 \times 2 37-50 μm Corasil RP C18; B 20 \times 4 25-40 μm Lichrosorb RP-8 + 250 \times 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: cefoxitin, cephalixin, cephaloridine

Noninterfering: alclofenac, aspirin, caffeine, cefadroxil, cefamandole, cefazolin, cefoperazone, cefotiam, cephalothin, diclofenac, ibuprofen, indomethacin, ketoprofen, lonazolac, mefenamic acid, naproxen, phenylbutazone, piroxicam

KEY WORDS

plasma; column-switching; rat; human

REFERENCE

Lee, Y.J.; Lee, H.S. Simultaneous determination of cefoxitin, cefuroxime, cephalixin and cephaloridine in plasma using HPLC and a column-switching technique. *Chromatographia*, **1990**, *30*, 80–84

SAMPLE

Matrix: blood, ear fluid

Sample preparation: 50 μL Plasma or ear effusion + 50 μL 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 \times 2.1 5 μm Hypersil C18

Column: 250 \times 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: 5.9

Internal standard: cefuroxime

OTHER SUBSTANCES

Extracted: cefpodoxime

KEY WORDS

plasma; chinchilla; middle ear effusion; cefuroxime is IS

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, urine

Sample preparation: Plasma. 200 μ L Plasma + 20 μ L 0.45 N phosphoric acid + 100 μ L methanol + 20 μ L 270 μ mol/L cephalexin, vortex 15 s, centrifuge for 3 min, remove 100 μ L supernatant, inject 20 μ L. Urine. 10 μ L Urine + 0.5 mL water + 20 μ L 270 μ mol/L cephalexin, vortex 15 s, remove 100 μ L supernatant, inject 20 μ L.

HPLC VARIABLES

Guard column: 100 \times 4.7 Co:Pell ODS

Column: 150 \times 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: 13

Internal standard: cephalexin

Limit of detection: 1 nmol/mL (plasma); 50 nmol/mL (urine)

OTHER SUBSTANCES

Simultaneous: cefotiam

KEY WORDS

plasma

REFERENCE

Lecaillon, J.B.; Rouan, M.C.; Soupart, 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: cell suspensions

Sample preparation: 100 μ L Cell suspension + 100 μ L cefoperazone solution + 100 μ L Hanks balanced salt solution, sonicate 30 min, add 800 μ L MeCN, centrifuge at 13000 g

for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μ L mobile phase, inject 75 μ L.

HPLC VARIABLES

Column: μ Bondapak 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: 6.8

Internal standard: cefoperazone

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: Dilute 1 mL to 10 mL with water, add 3-6 mL to 20 mL 1.5 mg/mL 5-methylresorcinol in water, make up to 100 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrasphere C18

Mobile phase: MeCN:100 mM pH 3.4 acetate buffer 1:10 (Buffer was 50 mL 100 mM sodium acetate diluted to 1 L with 100 mM acetic acid.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Internal standard: 5-methylresorcinol

OTHER SUBSTANCES

Simultaneous: aminophylline, theophylline

KEY WORDS

injections; 5% dextrose; saline; stability-indicating

REFERENCE

Stewart, J.T.; Warren, F.W.; Johnson, S.M. Stability of cefuroxime sodium and aminophylline or theophylline. *Am.J.Hosp.Pharm.*, **1994**, 51, 809-811

SAMPLE

Matrix: solutions

Sample preparation: Dilute 1:100 with water.

HPLC VARIABLES

Column: Novapak C18 (model no. PN86344)

Mobile phase: MeCN:glacial acetic acid:water 6:1:93, adjusted to a pH of 4.0

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM**Retention time:** 5.4

OTHER SUBSTANCES**Interfering:** ceftazidime

KEY WORDSwater; stability-indicating

REFERENCE

Stiles, M.L.; Allen, L.V., Jr.; Fox, J.L. Stability of ceftazidime (with arginine) and of cefuroxime sodium in infusion-pump reservoirs. *Am.J.Hosp.Pharm.*, **1992**, *49*, 2761-2764

SAMPLE**Matrix:** solutions**Sample preparation:** Separate the buffer containing the 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 10:90**Flow rate:** 1.5**Injection volume:** 10-20**Detector:** UV 270

OTHER SUBSTANCES**Also analyzed:** cefotaxime, cephacetrile

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:** Add 100 µL solution to 1 mL 1 mg/mL cefsulodin in water, vortex for 15 s, inject a 10 µL aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 10 µm Alltech C8**Mobile phase:** MeOH:10 mM sodium acetate 30:70 containing 1.7 g/L tetrabutylammonium hydrogen sulfate, pH adjusted to 6.5 with 5 M NaOH**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 10.0**Internal standard:** cefsulodin (4.0)

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

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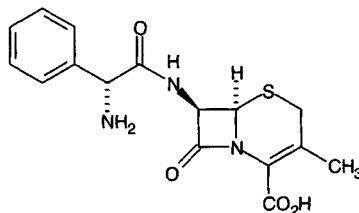
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- Fauzi, M.A.; Dine, T.; Luyckx, M.; Gressier, B.; Brunet, C.; Goudaliez, F.; Mallevais, M.L.; Cazin, M.; Cazin, J.C. Stability and compatibility studies of cefaloridine, cefuroxime and ceftazidime with PVC infusion bags. *Pharmazie*, **1994**, *49*, 425-427 [simultaneous cefaloridine, ceftazidime; saline; 5% dextrose]
- Wang, D.; Notari, R.E. Cefuroxime hydrolysis kinetics and stability predictions in aqueous solution. *J.Pharm.Sci.*, **1994**, *83*, 577-581 [simultaneous degradation products]
- Zhang, H.; Stewart, J.T. Determination of a cefuroxime and aminophylline/theophylline mixture by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1994**, *17*, 1327-1335 [orcinol (IS)]
- Kim, M.; Stewart, J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α -phenylcinnamionitrile quaternary ammonium salt as a new fluorescent ion-pair reagent. *J.Liq.Chromatogr.*, **1990**, *13*, 213-237 [derivatization; fluorescence detection; also benzoic acid, flufenamic acid, ibuprofen, ketoprofen, mefenamic acid, probenecid, salicylic acid, valproic acid]
- Das Gupta, V.; Stewart, K.R. Stability of cefuroxime sodium in some aqueous buffered solutions and intravenous admixtures. *J.Clin.Hosp.Pharm.*, **1986**, *11*, 47-54 [5% dextrose; saline; stability-indicating; simultaneous degradation products; cefazolin (IS)]
- Sanders, C.A.; Moore, E.S. Liquid-chromatographic assay of cefuroxime in plasma. *Clin.Chem.*, **1986**, *32*, 2109
- Campbell, C.J.; Langley, C. Measurement of rat-intestinal cefuroxime axetil esterase activity: comparison of an h.p.l.c. and coupled-enzyme assay. *Xenobiotica*, **1985**, *15*, 1011-1019 [enzyme incubations; cefuroxime determined]
- Coomber, P.A.; Jefferies, J.P.; Woodford, J.D. High-performance liquid chromatographic determination of cefuroxime. *Analyst*, **1982**, *107*, 1451-1456
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- Hekster, Y.A.; Baars, A.M.; Vree, T.B.; Van Klingerren, B.; Rutgers, A. Comparison of high performance liquid chromatography and microbiological assay in the determination of plasma cefuroxime concentrations in rabbits. *J.Antimicrob.Chemother.*, **1980**, *6*, 65-71

Cephalexin

Molecular formula: C₁₆H₁₇N₃O₄S

Molecular weight: 347.4

CAS Registry No.: 15686-71-2, 23325-78-2 (monohydrate),
105879-42-3 (HCl)



SAMPLE

Matrix: blood

Sample preparation: 50 μ L Plasma + 100 μ L 50 mM pH 7.0 phosphate buffer, mix. Inject 100 μ L onto column A and elute to waste with mobile phase A, after 5 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 for 4 min before the next injection.

HPLC VARIABLES

Column: A 20 \times 3.9 25-40 μ m LiChrosorb RP-8; B 10 \times 4 Nova-Pak C8 guard column + 250 \times 4.6 5 μ m Ultracarb 5 ODS-30 (Phenomenex)

Mobile phase: A 50 mM pH 7.0 phosphate buffer; B Gradient. A was MeCN:20 mM pH 7.0 phosphate buffer 4:96. B was MeCN:20 mM pH 7.0 phosphate buffer 30:70. A:B 55:45 for 10 min, to 0:100 over 8 min, maintain at 0:100 for 12 min.

Flow rate: 1

Injection volume: 100

Detector: UV 230

CHROMATOGRAM

Retention time: 7.4

Internal standard: cephalixin

OTHER SUBSTANCES

Extracted: ampicillin, metampicillin

Noninterfering: acetaminophen, caffeine, ibuprofen, phenobarbital, sulbactam

KEY WORDS

plasma; rat; column-switching; cephalixin is IS

REFERENCE

Lee, H.; Lee, J.S.; Lee, H.S. Simultaneous determination of ampicillin and metampicillin in biological fluids using high-performance liquid chromatography with column switching. *J.Chromatogr.B*, **1995**, *664*, 335-340

SAMPLE

Matrix: blood

Sample preparation: Mix 100 μ L plasma + 300 μ L 5 μ g/mL cefotaxime in pH 3.5 10 mM acetate buffer and keep at 4°. Inject 100 μ L 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 \times 2 37-50 μ m Corasil RP C18; B 20 \times 4 25-40 μ m Lichrosorb RP-8 + 250 \times 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: 6.2

Internal standard: cefotaxime

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: cefoxitin, cefuroxime, cephaloridine

Noninterfering: alclofenac, aspirin, caffeine, cefadroxil, cefamandole, cefazolin, cefoperazone, cefotiam, cephalothin, diclofenac, ibuprofen, indomethacin, ketoprofen, lonazolac, mefenamic acid, naproxen, phenylbutazone, piroxicam

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

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

Internal standard: coumarin-3-carboxylic acid (13)

OTHER SUBSTANCES

Extracted: cefaclor, cefazolin, ceftizoxime

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. *Clin.Chim.Acta*, **1990**, *190*, 121–123

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma + 70 μ L 1.2 M perchloric acid + 70 μ L 500 mM sodium heptanesulfonate, vortex 15 s, centrifuge for 3 min, inject a 5 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 40 \times 1 Co:Pell ODS

Column: 150 \times 1 5 μ m Nucleosil C18 in a glass-lined stainless steel column

Mobile phase: MeOH:2 mM phosphoric acid 26:74

Flow rate: 0.05

Injection volume: 5

Detector: UV 254 (2.4 μ L flow cell)

CHROMATOGRAM

Retention time: 20

Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Extracted: cefroxadin

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: 100 μ L Serum + 10 μ L 5 μ g/mL cefixime in MeOH + 100 μ L 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 μ L mobile phase, inject a 50-80 μ L aliquot.

HPLC VARIABLES

Guard column: RCSS Silica Guard Pak (Waters)

Column: 150 \times 4.6 5 μ m Ultrasphere Octyl C8

Mobile phase: MeOH:12.5 mM pH 2.6 NaH₂PO₄ (pH adjusted with concentrated phosphoric acid) 20:80

Flow rate: 2

Injection volume: 50-80

Detector: UV 240

CHROMATOGRAM

Retention time: 15

Internal standard: cefixime (11)

Limit of detection: 1 μ g/ mL

OTHER SUBSTANCES

Extracted: cefaclor, cefadroxil, cephadrine

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 cephadrine—in human serum. *Clin.Chem.*, **1987**, *33*, 1788–1790

SAMPLE

Matrix: blood

Sample preparation: 300 μ L Plasma + 300 μ L 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 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 10 μ m C18

Column: 300 \times 4 10 μ m μ Bondapak 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: 8-chlorotheophylline (4)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: cefoperazone, cefotaxime

Interfering: cefoxitin, cephaloridine

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: 500 μ L 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 2 mL buffer, add 1 mL 0.5% hydrogen peroxide in buffer, heat in a boiling water bath for 70 min, cool to room temperature, add 2 mL 500 mM 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 μ L MeOH containing IS, inject a 20 μ L 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 \times 4 5 μ m 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 anthranilate (9 (?))

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Interfering: ampicillin, cephradine

KEY WORDS

plasma; derivatization; rat; pharmacokinetics

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: 200 μ L Plasma + 70 μ L 10% trichloroacetic acid, vortex 15 s, centrifuge for 3 min, remove 100 μ L supernatant, inject 20 μ L.

HPLC VARIABLES

Guard column: 100 \times 4.7 Co:Pell ODS

Column: 100 \times 7.5 LiChrosorb RP-8

Mobile phase: MeOH:2 mM phosphoric acid 28:72

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 19

Limit of detection: 1 nmol/mL

KEY WORDS

plasma

REFERENCE

Lecaillon, J.B.; Rouan, M.C.; Soupart, 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: blood, urine

Sample preparation: Plasma or serum. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH then 2 mL water, do not allow to go dry. 500 μ L Plasma or serum + 100 μ L water + 50 μ L 25% acetic acid, mix, add to SPE cartridge, wash with two 1 mL portions of water, elute with 3 mL MeOH. Evaporate eluate under nitrogen, add 200 μ L mobile phase, vortex, inject a 25 μ L aliquot. Urine. Dilute 100:1 (ratio may vary depending on concentration) with water, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-18-DB

Mobile phase: MeOH:THF:buffer 16:4:80 (Buffer was 1 g sodium 1-heptanesulfonate + 15 mL triethylamine in 1 L water with the pH adjusted to 2.3 with concentrated phosphoric acid.)

Column temperature: 30

Flow rate: 1.4

Injection volume: 25-50

Detector: UV 265

CHROMATOGRAM

Retention time: 9.2

Internal standard: cephalixin

OTHER SUBSTANCES

Extracted: cefaclor, hydroxyloracarbef, loracarbef

Noninterfering: acetaminophen, caffeine

KEY WORDS

plasma; serum; SPE; pharmacokinetics; cephalixin is IS

REFERENCE

Kovach, P.M.; Lantz, R.J.; Brier, G. High-performance liquid chromatographic determination of loracarbef, a potential metabolite, cefaclor and cephalixin in human plasma, serum and urine. *J.Chromatogr.*, **1991**, *567*, 129-139

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 150 μ L 10% trichloroacetic acid + 500 μ L MeCN, mix, add 1.5 mL dichloromethane, vortex, centrifuge, inject a 60 μ L aliquot of the aqueous supernatant. Urine. 5 mL Urine + 5 mL 20 mM pH 3.8 sodium acetate buffer, mix. 500 μ L Buffered urine + 150 μ L 5% trichloroacetic acid, vortex, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 45 \times 4 37-50 μ m C18/Corasil

Column: Zorbax C8 (plasma) or Partisil 5 ODS-3 RAC C18 (urine)

Mobile phase: MeCN:glacial acetic acid:water 17:2:81 (plasma) or MeCN:MeOH:THF:trichloroacetic acid:glacial acetic acid:sodium acetate trihydrate:sodium dodecyl sulfate:water 25:3:0.925:0.075:0.25:0.077:0.134:70.75 (v/v/v/w/v/w/w/v)

Flow rate: 1.5 (plasma) or 2 (urine)

Injection volume: 10-60

Detector: UV 280

CHROMATOGRAM

Retention time: 17 (plasma), 23 (urine)

Internal standard: cephalixin

OTHER SUBSTANCES

Extracted: cefprozil

KEY WORDS

plasma; cephalixin is IS; human; rat; pharmacokinetics

REFERENCE

Shyu, W.C.; Shukla, U.A.; Shah, V.R.; Papp, E.A.; Barbhaiya, R.H. Simultaneous high-performance liquid chromatographic analysis of cefprozil diastereomers in a pharmacokinetic study. *Pharm.Res.*, **1991**, *8*, 992-996

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: 1.4 ng/mL (F); 1.9 ng/mL (UV)

OTHER SUBSTANCES

Also analyzed: cefaclor, cefroxadine, cephradine

Noninterfering: amidopyrin, aspirin, barbital, caffeine, cefmenoxime, cefotaxime, ceftizoxime, ceftriaxone, cetazidime, diazepam, dibekacin, gentamycin, kanamycin, lidocaine, neltimicin, tetracaine, theophylline, tobramycin

KEY WORDS

post-column reaction; plasma; F detection may be less susceptible to interferences

REFERENCE

Blanchine, 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: blood, urine

Sample preparation: Serum. 200 μL Serum + 250 μL 4 μg/mL ceftazidime in 6% perchloric acid, vortex 15 s, centrifuge at 1500 g for 15 min, inject 25 μL supernatant. Urine. 250 μL Urine + 1 mL 4 μg/mL ceftazidime in 6% perchloric acid, mix, inject 10 μL supernatant.

HPLC VARIABLES

Guard column: 30 × 2 30-38 μm CO:Pell ODS

Column: 150 × 4.6 5 μm Pecosphere C18

Mobile phase: 100 mL MeCN + 90 mL 0.5 M (NH₄)H₂PO₄ adjusted to pH 3.0 with 10% phosphoric acid. This mixture was made up to 1 L with water.

Flow rate: 1.7

Injection volume: 10-25

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Internal standard: ceftazidime

OTHER SUBSTANCES

Noninterfering: amikacin, carbenicillin, cefazolin, cefoperazone, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, moxalactam, oxacillin, ticarcillin, tobramycin, vancomycin

Interfering: cefatoxime, cefonicid, methicillin, penicillin G

KEY WORDS

serum

REFERENCE

Emm, T.A.; Leslie, J.; Chai, M.; Lesko, L.J.; Perkal, M.B. High-performance liquid chromatographic assay of cephalexin in serum and urine. *J.Chromatogr.*, **1988**, *427*, 162–165

SAMPLE**Matrix:** bulk**Sample preparation:** Dissolve in water at a concentration of 5 mg/mL, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m YMC-ODS (YMC)**Mobile phase:** Gradient. A was 50 mM sodium dihydrogen phosphate adjusted to pH 4.0 with phosphoric acid. B was MeCN:50 mM sodium dihydrogen phosphate adjusted to pH 4.0 with phosphoric acid 45:55. A:B from 95:5 to 75:25 over 30 min, to 0:100 over 15 min, maintain at 0:100 for 5 min, return to 95:5 and equilibrate for 14 min.**Flow rate:** 1**Injection volume:** 20**Detector:** UV 220

CHROMATOGRAM**Retention time:** 28

OTHER SUBSTANCES**Simultaneous:** impurities, excipients**Also analyzed:** cefaclor

REFERENCE

Olsen, B.A.; Baertschi, S.W.; Riggan, R.M. Multidimensional evaluation of impurity profiles for generic cephalexin and cefaclor antibiotics. *J.Chromatogr.*, **1993**, *648*, 165–173

SAMPLE**Matrix:** bulk, formulations**Sample preparation:** Weigh out bulk drug, capsule contents, granules, or powders equivalent to 35 mg cephalexin, add 1 mL 2.52 mg/mL acetaminophen in MeOH:water 20:80, make up to 50 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeOH:1.25% acetic acid 25:75**Flow rate:** 1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 11.3**Internal standard:** acetaminophen (6)

OTHER SUBSTANCES**Simultaneous:** degradation products

KEY WORDS

capsules; granules; powder

REFERENCE

Hsu, M.C.; Lin, Y.-S.; Chung, H.-C. High-performance liquid chromatographic method for potency determination of cephalexin in commercial preparations and for stability studies. *J.Chromatogr.A*, **1995**, *692*, 67–72

SAMPLE

Matrix: bulk, formulations

Sample preparation: Dissolve in water to a concentration of 70 $\mu\text{g/mL}$, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeOH:water:acetic acid 30:70:0.1

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 10

Limit of quantitation: 5.6 $\mu\text{g/mL}$

OTHER SUBSTANCES

Simultaneous: impurities, cefaclor, cefadroxil, cefamandole, cefamandole nafate, cefazolin, cefoperazone, cefotaxime, cefoxitin, ceftizoxime, cephalothin, cephalirin, cephradine

REFERENCE

Ting, S. Reverse-phase liquid chromatographic analysis of cephalosporins. *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130

SAMPLE

Matrix: perfusate

Sample preparation: Filter, inject an aliquot.

HPLC VARIABLES

Column: 5 μm Ultrasphere C18

Mobile phase: MeOH:50 mM pH 5 sodium phosphate buffer 20:80

Detector: UV 220

CHROMATOGRAM

Retention time: 9

REFERENCE

Hu, M.; Zheng, L.; Chen, J.; Liu, L.; Zhu, Y.; Dantzig, A.H.; Stratford, R.E., Jr. Mechanisms of transport of quinapril in Caco-2 cell monolayers: Comparison with cephalexin. *Pharm.Res.*, **1995**, *12*, 1120–1125

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 220 \times 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: 4.5

OTHER SUBSTANCES

Simultaneous: 7-aminocephaloropropanoic acid, 7-aminodesacetoxycephalosporanic acid, cefazolin, cephaloridine, cephalothin, cephadrine

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: 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: 9

OTHER SUBSTANCES

Simultaneous: amoxicillin, ampicillin, cefaclor, cephadrine

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

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: 8.5

OTHER SUBSTANCES

Simultaneous: 7-aminocephalosporanic acid, cefadroxil, cefazolin, cefotaxime, cephaloridine, cephalosporin C, cephalothin, cephalirin, 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:** 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 + 50 μ L 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 μ L aliquot of the eluate.

HPLC VARIABLES**Guard column:** Nova-Pak C18 guard column**Column:** 250 \times 4.6 5 μ m Econosphere C18**Mobile phase:** MeOH:20 mM NaH₂PO₄ 23:77, pH 5.0**Flow rate:** 1**Injection volume:** 100**Detector:** UV 270

CHROMATOGRAM**Retention time:** 11.5**Internal standard:** cephalexin

OTHER SUBSTANCES**Extracted:** cefazolin

KEY WORDS

rat; liver; spleen; lung; SPE; cephalexin is IS

REFERENCELiang, D.; Chow, D.; White, C. High-performance liquid chromatographic assay of cefazolin in rat tissues. *J. Chromatogr. B*, **1994**, 656, 460–465

SAMPLE**Matrix:** tissue**Sample preparation:** Muscle, fat. 10 g Minced tissue + 1 mL cephadrine in 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 μ m), 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 cephadrine in 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 μ m). 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 μ L 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 \times 4 25-40 μ m LiChroprep RP 18; B 4 \times 4 5 μ m LiChrospher 100 CH-18 +250 \times 4 5 μ m 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 system with 60 mL MeOH:water 30:70 and 30 mL MeOH.)**Flow rate:** 1

Injection volume: 2000**Detector:** UV 260**CHROMATOGRAM****Retention time:** 11**Internal standard:** cephradine (15)**Limit of quantitation:** 45 ng/g**KEY WORDS**

cow; muscle; fat; liver; kidney

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

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- Granero, L.; Gimeno, M.J.; Torres-Molina, F.; Chesa-Jiménez, J.; Peris, J.E. Studies on the renal excretion mechanisms of cefadroxil. *Drug Metab.Dispos.*, **1994**, *22*, 447–450 [rat; plasma; urine; gradient; extracted cefadroxil; LOD 300 ng/mL]
- Muranushi, N.; Horie, K.; Masuda, K.; Hirano, K. Characteristics of ceftibuten uptake into Caco-2 cells. *Pharm.Res.*, **1994**, *11*, 1761–1765 [also cefaclor, cefadroxil, cefazolin, cephalexin, cephradine, cycloxacillin, latamoxef]
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- Parker, C.E.; Perkins, J.R.; Tomer, K.B.; Shida, Y.; O'Hara, K. Nanoscale packed capillary liquid chromatography-electrospray ionization mass spectrometry: analysis of penicillins and cepheims. *J.Chromatogr.*, **1993**, *616*, 45–57 [serum; also amoxicillin, ampicillin, carbenicillin, cefalothin, cefazolin, cefmenoxime, cefmetazole, cefoperazone, cefotaxime, cefotiam, cefoxitin, cloxacillin, dicloxacillin, penicillin G, piperacillin, sulbenicillin]
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- Wang, D.P.; Yeh, M.K. Stability-indicating method for cephalexin in capsules by high-performance liquid chromatography. *Chung-hua Yao Hsueh Tsa Chih.*, **1990**, *42*, 349–353
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- Najib, N.M.; Suleiman, M.S.; El-Sayed, Y.M.; Abdulhameed, M.E. High performance liquid chromatographic analysis of cephalexin in serum and urine. *J.Clin.Pharm.Ther.*, **1987**, *12*, 419–426
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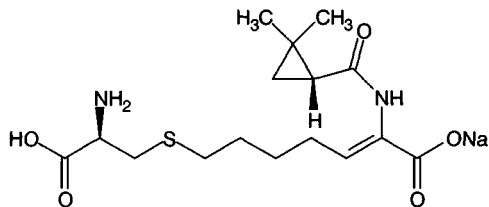
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Cilastatin

Molecular formula: C₁₆H₂₆N₂O₅S

Molecular weight: 358.5

CAS Registry No.: 82009-34-5 (cilastatin),
81129-83-1 (cilastatin sodium)



SAMPLE

Matrix: bile, blood, urine

Sample preparation: Plasma, urine. Condition a 3 mL 200 mg C8 Bond Elut SPE cartridge with 1 mL MeOH and 2 mL water. 100-400 μ L Plasma or 10 μ L urine + 10 μ L 1 mg/mL N-propionylcilastatin in MeCN + 1 mL water, acidify with 1 drop concentrated HCl, vortex, add to SPE cartridge, elute with 1 mL MeOH:water 80:20. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 50-100 μ L aliquot. Bile. 10 μ L Bile + 1 mL mobile phase + 10 μ L 1 mg/mL N-propionylcilastatin in MeCN, vortex for 30 s, centrifuge at 1500 g for 5 min, inject a 50-100 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 5 μ m Brownlee RP-18

Column: 100 \times 4.6 5 μ m Partisil ODS-3 RAC 2

Mobile phase: MeCN:50 mM NaH₂PO₄ containing 5 mM PIC B-8 (Waters), pH adjusted to 4.0 with 85% phosphoric acid 12:88

Flow rate: 1.2

Injection volume: 50-100

Detector: UV 210

CHROMATOGRAM

Retention time: 4.6

Internal standard: N-propionylcilastatin (15.1)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: N-acetylcilastatin

KEY WORDS

plasma; rat; SPE

REFERENCE

Chen, I.-W.; Hsieh, J.Y.-K.; Lin, J.H.; Duggan, D.E. High-performance liquid chromatographic determination of cilastatin and its major metabolite N-acetylcilastatin in rat plasma, urine and bile. *J.Chromatogr.*, **1990**, 534, 119-126

SAMPLE

Matrix: blood

Sample preparation: Stabilize plasma with an equal volume of ethylene glycol:1 M pH 6.0 morpholineethanesulfonate buffer 50:50.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Adsorbosphere C8

Mobile phase: MeOH:buffer:water 4:10:86 (Buffer was 20.9 g 3-(N-morpholino)propanesulfonic acid in 1 L water, pH 7.0.)

Column temperature: 50

Flow rate: 4

Injection volume: 10

Detector: UV 245

KEY WORDS

plasma; dog; pharmacokinetics

REFERENCE

Wong, J.; Kuu, W.-Y.; Burke, R.; Johnson, R.; Wood, R.W. Comparison of simulated and in-vivo plasma levels of cilastatin following intravenous in-line drug administration. *Pharm.Res.*, **1995**, *12*, 144-148

SAMPLE

Matrix: blood

Sample preparation: Dilute serum with an equal volume of 500 mM pH 6.0 2-[N-morpholine]ethanesulfonic acid (MES) buffer, filter (Amicon Centrifree with YMT membrane) a 700 μ L aliquot while centrifuging at 1000-2000 g for 30 min, vortex filtrate, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 40 \times 4 40 μ m pellicular reverse-phase (Vydac)

Column: 200 \times 4 Micro Pak MCH 10 reverse-phase

Mobile phase: MeOH:100 mM pH 2.5 potassium phosphate buffer 24:76 (Wash with 50:50 at the end of each run.)

Column temperature: 30

Flow rate: 1

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Retention time: 20

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: caffeine, carbenicillin, ceftazidime, chloramphenicol, phenobarbital, phenytoin, salicylic acid, sulfamethoxazole, theophylline, ticarcillin

Noninterfering: acetaminophen, cimetidine, imipenem, moxalactam, theobromine

KEY WORDS

serum; pharmacokinetics

REFERENCE

Myers, C.M.; Blumer, J.L. Determination of imipenem and cilastatin in serum by high-pressure liquid chromatography. *Antimicrob.Agents Chemother.*, **1984**, *26*, 78-81

SAMPLE

Matrix: blood, tissue

Sample preparation: Serum. 500 μ L Serum + 500 μ L MeOH, vortex vigorously, heat at 35° for 15 min, centrifuge at 4° at 4000 rpm for 10 min, inject a 20 μ L aliquot of the supernatant. Tissue. Homogenize tissue with pH 7.2 sodium borate buffer, centrifuge at 4° at 4000 rpm for 10 min, remove a 500 μ L aliquot of the supernatant and add it to 500 μ L MeOH, vortex vigorously, heat at 35° for 15 min, centrifuge at 4° at 4000 rpm for 10 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: C18 Corasil

Column: 300 mm long μ Bondapak C18

Mobile phase: Isopropanol:water 7:93, adjusted to pH 3 with phosphoric acid

Flow rate: 1.5

Injection volume: 20

Detector: UV 210

CHROMATOGRAM

Retention time: 11.50

Limit of detection: 500 ng/mL

KEY WORDS

serum

REFERENCE

Krausse, R.; Ullmann, U. Determination of imipenem and cilastatin in serum and tissue by high-pressure liquid chromatography. *Infection*, **1986**, *14*, 243-245

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 75 μ L 30 μ g/mL IS + 2 mL 500 mM pH 3 KH_2PO_4 , vortex, add to an activated Sep-Pak C18 SPE cartridge, wash with 20 mL 1 mM orthophosphoric acid, elute with 1.5 mL MeOH, add the eluate to 1 mL water, vortex, inject a 50-200 μ L aliquot. Urine. Stabilize urine by mixing with an equal volume of 1 M pH 6.8 MOPS buffer:ethylene glycol 50:50. 1 mL Stabilized urine + 50 μ L 100 μ g/mL IS + 2.5 mL 20 mM orthophosphoric acid, vortex, add to an activated Sep-Pak C18 SPE cartridge, wash with 20 mL 1 mM orthophosphoric acid, elute with 1.5 mL MeOH, add the eluate to 1 mL water, vortex, inject a 25-75 μ L aliquot.

HPLC VARIABLES

Guard column: 40 \times 4.6 ODS-10 (Bio-Rad)

Column: 250 \times 4.6 Bio-Sil ODS-10 (Bio-Rad)

Mobile phase: Isopropanol:0.2% orthophosphoric acid 10.9:89.1, pH 3 (plasma) or isopropanol:0.2% orthophosphoric acid 6:94, pH 3 (urine)

Flow rate: 2

Injection volume: 25-200

Detector: F ex 335 em 455 following post-column reaction with o-phthalaldehyde reagent solution (Pierce) pumped at 1 mL/min. The mixture flowed through a 250 \times 4.6 column packed with 40 μ m glass beads (Whatman) to the detector.

CHROMATOGRAM

Retention time: 5.04 (plasma), 7.95 (urine)

Internal standard: S-(p-methylbenzyl)-L-cysteine (8.04 (plasma), 12.01 (urine))

Limit of detection: 750 ng/mL (plasma); 2500 ng/mL (urine)

OTHER SUBSTANCES

Noninterfering: metabolites, imipenem

KEY WORDS

plasma; derivatization; post-column reaction; SPE

REFERENCE

Demetriades, J.L.; Souder, P.R.; Entwistle, L.A.; Vincek, W.C.; Musson, D.G.; Bayne, W.F. High-performance liquid chromatographic determination of cilastatin in biological fluids. *J.Chromatogr.*, **1986**, *382*, 225-231

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 150 × 4.6 5 μm Adsorbosphere C8

Mobile phase: MeOH:water:100 mM pH 7 3-[N-morpholino]propanesulfonic acid buffer
4:86:10

Detector: UV 245

OTHER SUBSTANCES

Simultaneous: imipenem

KEY WORDS

injections; saline; 5% dextrose

REFERENCE

Jenke, D.R. Drug binding by reservoirs in elastomeric infusion devices. *Pharm.Res.*, **1994**, *11*, 984–989

SAMPLE

Matrix: formulations

Sample preparation: Dilute 1:100 with mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 200 × 4.6 RP-8 (Hewlett-Packard)

Mobile phase: MeCN:MeOH:buffer 0.4:0.5:99.1, adjusted to pH 7.00 with NaOH (Buffer was 4 mM 3-[N-morpholino]propanesulfonic acid (MOPS) containing 2 g/L sodium hexane sulfate.)

Flow rate: 1.8

Injection volume: 30

Detector: UV 250

CHROMATOGRAM

Retention time: 8.8

OTHER SUBSTANCES

Extracted: imipenem

KEY WORDS

injections; total parenteral nutrition; stability-indicating

REFERENCE

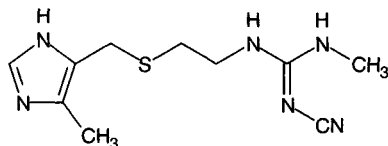
Zaccardelli, D.S.; Krcmarik, C.S.; Wolk, R.; Khalidi, N. Stability of imipenem and cilastatin sodium in total parenteral nutrient solution. *J.Parenter.Enteral.Nutr.*, **1990**, *14*, 306–309

Cimetidine

Molecular formula: C₁₀H₁₆N₆S

Molecular weight: 252.3

CAS Registry No.: 51481-61-9, 70059-30-2 (HCl)



SAMPLE

Matrix: blood

Sample preparation: 250 μ L Plasma + 25 μ L 2.5 μ g/mL IS in MeOH:water 50:50 + 20 μ L 2.5 M NaOH + 100 μ L saturated potassium carbonate, vortex for 1 min, add 1 mL ethyl acetate, vortex for 90 s, centrifuge at 9500 g. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μ L water, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Nucleosil C18

Mobile phase: MeCN:10 mM pH 6.2 phosphate buffer 25:75, containing 2.5 g/L heptane-sulfonic acid

Flow rate: 0.9

Injection volume: 50

Detector: UV 228

CHROMATOGRAM

Retention time: 6.3

Internal standard: BN CK249 (Glaxo) (7.1)

Limit of detection: 15 ng/mL

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kelly, M.T.; McGuirk, D.; Bloomfield, F.J. Determination of cimetidine in human plasma by high-performance liquid chromatography following liquid-liquid extraction. *J.Chromatogr.B*, **1995**, 668, 117-123

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 50 μ L 2 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 850 g 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 μ L 2.5 μ g/mL 1,3-dimethyluric acid in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: reverse-phase

Mobile phase: MeOH:10 mM pH 5.2 sodium acetate 25:75

Flow rate: 1.2

Injection volume: 20

Detector: UV 228

CHROMATOGRAM

Internal standard: 1,3-dimethyluric acid

KEY WORDS

plasma; rat

REFERENCE

Nagai, N.; Furuhashi, M.; Ogata, H. Drug interactions between theophylline and H₂-antagonists, roxatidine acetate hydrochloride and cimetidine: Pharmacokinetic analysis in rats in vivo. *Biol.Pharm.Bull.*, **1995**, *18*, 1610-1613

SAMPLE

Matrix: blood

Sample preparation: 300 μ L Plasma + 150 μ L water + 300 μ L 2.5 M NaOH + 2 μ g codeine (dissolved in water), extract with 5 mL dichloromethane. Remove the organic layer and evaporate it to dryness at 37°, reconstitute the residue in 200 μ L mobile phase, inject an 80 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μ m Spheri-10 RP-18 (Brownlee)

Column: 250 \times 4.6 10 μ m Partisil ODS/10

Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM KH₂PO₄ adjusted to pH 2.7 with 1 M HCl.)

Flow rate: 2

Injection volume: 80

Detector: UV 228

CHROMATOGRAM

Retention time: 6-7

Internal standard: codeine (10-11)

Limit of detection: 100 ng/mL

Limit of quantitation: 250 ng/mL

KEY WORDS

dog; plasma; pharmacokinetics

REFERENCE

Langguth, P.; Lee, K.M.; Spahn-Langguth, H.; Amidon, G.L. Variable gastric emptying and discontinuities in drug absorption profiles: Dependence of rates and extent of cimetidine absorption on motility phase and pH. *Biopharm.Drug Dispos.*, **1994**, *15*, 719-746

SAMPLE

Matrix: blood

Sample preparation: Mix 1 mL plasma + 0.1 mL 20 μ g/mL IS in water, add to 1 mL Baker C-18 column, rinse twice with 1 mL water, elute with 0.5 mL MeOH. In each case passage of fluid through the column is helped by centrifugation.

HPLC VARIABLES

Column: 250 \times 4 5 μ m LiChrosorb RP-18

Mobile phase: MeOH:10 mM pH 8.9 ammonium carbamate 40:60

Column temperature: 45

Flow rate: 1.2

Injection volume: 25

Detector: UV 220

CHROMATOGRAM

Retention time: 4

Internal standard: N-cyano-N'-(2-[(5-methyl-1H-imidazol-4-yl)-methylthio]ethyl)-S-methyl-isothourea

KEY WORDS

plasma

REFERENCE

Nitsche, V.; Mascher, H. New rapid assay of cimetidine in human plasma by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1983**, *273*, 449–452

SAMPLE

Matrix: blood, gastric fluid

Sample preparation: Plasma (human). 250 μ L Plasma + 150 μ L 8 μ g/mL IS in MeOH + 25 μ L 5 M NaOH, vortex, add 2 mL MeCN, shake 5 min, centrifuge at 4000 g for 10 min. Remove organic layer and add to 1 mL 20 mM HCl saturated with NaCl (300 g/L). Shake 10 min, centrifuge at 4000 g for 10 min. Remove aqueous layer and add it to 100 μ L 5 M NaOH + 2 mL MeCN. Shake 5 min, remove organic layer and evaporate under dry nitrogen in a water bath at 40°. Take up residue in 250 μ L 1 mM HCl in MeOH, inject 15 μ L. Gastric Fluid (monkey). 250 μ L Gastric fluid + 150 μ L 43 μ g/mL IS in MeOH + 2 mL 20 mM HCl saturated with NaCl (300 g/L), vortex, add 2 mL MeCN, shake 10 min, centrifuge at 4000 g for 10 min. Discard organic layer and extract again with 2 mL MeCN. Remove aqueous layer and add it to 100 μ L 5 M NaOH + 2 mL MeCN. Shake 10 min, remove organic layer and evaporate under dry nitrogen in a water bath at 40°. Take up residue in 250 μ L 1 mM HCl in MeOH, inject 15 μ L.

HPLC VARIABLES

Column: Waters RCM-100 radial compression cartridge

Mobile phase: MeOH:8.7 mM KH_2PO_4 + 3.04 mM Na_2HPO_4 (pH 7.41) 34:66 (w/w)

Flow rate: 3

Injection volume: 15

Detector: UV 228

CHROMATOGRAM

Retention time: 2.78

Internal standard: N-cyano-N'-methyl-N''-[3-(4-imidazolyl)propyl]-guanidine

Limit of detection: 200 ng/mL

KEY WORDS

plasma

REFERENCE

Abdel-Rahim, M.; Ezra, D.; Peck, C.; Lazar, J. Liquid-chromatographic assay of cimetidine in plasma and gastric fluid. *Clin.Chem.*, **1985**, *31*, 621–623

SAMPLE

Matrix: blood, milk

Sample preparation: 100 μ L Serum or whole milk + 25 μ L 2.5 μ g/mL ranitidine in MeOH + 4 mL dichloromethane, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 RP-18 (Applied Biosystems)

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:water:glacial acetic acid:triethylamine 15:85:0.15:0.02

Flow rate: 1

Injection volume: 50

Detector: UV 228

CHROMATOGRAM

Retention time: 7.8

Internal standard: ranitidine (10.7)

Limit of detection: 50 ng/mL

KEY WORDS

serum; whole milk; pharmacokinetics

REFERENCE

Oo, C.Y.; Kuhn, R.J.; Desai, N.; McNamara, P.J. Active transport of cimetidine into human milk. *Clin.Pharmacol.Ther.*, **1995**, *58*, 548–555

SAMPLE

Matrix: blood, CSF, tissue

Sample preparation: Blood. Hemolyze 25 μ L whole blood with 50 μ L water. 25 μ L Plasma or hemolyzed blood + 100 μ L 100 μ g/mL ranitidine + 100 μ L 5 M NaOH + 5 mL dichloromethane, mix, shake for 10 min, centrifuge at 1650 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L mobile phase, inject a 25 μ L aliquot. (To measure unbound cimetidine in plasma inject 25 μ L ultrafiltrate (Amicon MPS-3 centrifree).) Tissue. Brain tissue + 100 μ L 50 μ g/mL ranitidine + 1 mL saline, homogenize in an ice bath for 1 min, add 100 μ L 1 M NaOH, add 5 mL dichloromethane, extract. Remove 3 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L mobile phase, centrifuge at 10000 g, inject a 25 μ L aliquot. CSF. Inject an aliquot directly.

HPLC VARIABLES

Column: 250 \times 4 Senshu gel 7C18H (Senshu)

Mobile phase: MeCN:5 mM NaH₂PO₄ containing 5 mM tetramethylammonium chloride 5:95

Column temperature: 40

Flow rate: 2

Injection volume: 25

Detector: UV 225

CHROMATOGRAM

Internal standard: ranitidine

Limit of detection: 50 (CSF), 500 (brain), 1000 (blood) ng/g

KEY WORDS

rat; plasma; brain; ultrafiltrate; whole blood; pharmacokinetics

REFERENCE

Nakada, Y.; Yamamoto, K.; Kawakami, J.; Sawada, Y.; Iga, T. Effect of acute renal failure on neurotoxicity of cimetidine in rats. *Pharm.Res.*, **1995**, *12*, 1953–1957

SAMPLE

Matrix: blood, urine

Sample preparation: 200 μ L Serum or urine + 100 μ L 0.072 μ g/mL procaine hydrochloride (+ 100 μ L 6.25% NaHCO₃ solution for urine samples) + 5 mL dichloromethane, vortex 210 s. Evaporate organic phase to dryness under nitrogen, take up in 100 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Partisil-10 ODS-3

Mobile phase: MeCN:10 mM pH 4.8 potassium phosphate buffer 7:93

Flow rate: 2

Injection volume: 20

Detector: UV 228

CHROMATOGRAM**Retention time:** 6.2**Internal standard:** procaine**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES**Noninterfering:** acetaminophen, caffeine, cimetidine sulfoxide, diazepam, digoxin, flurazepam, furosemide, methyldopa, minoxidil, propranolol, quinidine, sulfinpyrazone**Interfering:** procainamide, tolazamide

KEY WORDSserum

REFERENCEGuay, D.R.; Bockbrader, H.N.; Matzke, G.R. High-performance liquid chromatographic analysis of cimetidine in serum and urine. *J.Chromatogr.*, **1982**, *228*, 398–403

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 NaH₂PO₄ 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 WORDSstability-indicating; injections; saline

REFERENCEMayron, 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:** Inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 10 μm Econosil C18**Mobile phase:** MeOH:water:phosphoric acid:sodium 1-hexanesulfonate 24:76:0.03:0.094**Flow rate:** 2**Injection volume:** 20**Detector:** UV 220

CHROMATOGRAM**Retention time:** 16.8

KEY WORDS

injections; saline; stability-indicating

REFERENCE

Ku, Y.-M.; Min, D.I.; Kumar, V.; Noormohamed, S.E. Compatibility of tacrolimus injection with cimetidine hydrochloride injection in 0.9% sodium chloride injection. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2024–2025

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 \times 300 $\mu\text{Bondapak C18}$

Mobile phase: MeCN:MeOH:10 mM pH 2.6-2.7 phosphate buffer 7:14:79, containing 5 mM tetrabutylammonium hydrogen sulfate

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 3.27

Internal standard: cimetidine

OTHER SUBSTANCES

Simultaneous: ampicillin, aztreonam, sulbactam

KEY WORDS

saline; injections; cimetidine is IS

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: injections

Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.8 Spherisorb S5CN

Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH_2PO_4 adjusted to pH 5.4 with 1 M NaOH.)

Flow rate: 1.5

Injection volume: 10

Detector: UV 216

CHROMATOGRAM

Retention time: 4.5

Internal standard: cimetidine

OTHER SUBSTANCES

Simultaneous: ondansetron

KEY WORDS

5% dextrose; cimetidine is IS

REFERENCE

Bosso, J.A.; Prince, R.A.; Fox, J.L. Compatibility of ondansetron hydrochloride with fluconazole, ceftazidime, aztreonam, and ceftazolin sodium under simulated Y-site conditions. *Am.J.Hosp.Pharm.*, 1994, 51, 389-391

SAMPLE

Matrix: perfusate

Sample preparation: 1 mL Perfusate + 100 μ L 20 μ g/mL procainamide + 1 mL 2 M NaOH + 6 mL ethyl acetate, vortex for 3 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 110 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 100 mm long 5 μ m Radpak C18

Mobile phase: MeCN:water:triethylamine 6:94:1, adjusted to pH 3 with concentrated phosphoric acid

Injection volume: 40

Detector: UV 228

CHROMATOGRAM

Internal standard: procainamide

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, urine

Sample preparation: Urine. Add 10 μ L urine diluted 10 times with 10 mM pH 7.5 Na₂HPO₄ buffer to 1-5 μ g nizatidine, make up volume to 300 μ L with 10 mM pH 7.5 Na₂HPO₄ buffer. Place solution on YM-10 ultrafiltration membrane with a cut-off of 10000, centrifuge at 4000 g for 20 min. Mix 180 μ L filtrate with 20 μ L MeOH, inject 50 μ L. Perfusate. Add 10-100 μ L perfusate to 1-5 μ g nizatidine, make up volume to 300 μ L with 10 mM pH 7.5 Na₂HPO₄ buffer. Place solution on YM-10 ultrafiltration membrane with a cut-off of 10 000, centrifuge at 4000 g for 20 min. Mix 180 μ L filtrate with 20 μ L MeOH, inject 50 μ L.

HPLC VARIABLES

Guard column: 75 \times 2.1 5 μ m LiChrosorb RP-18

Column: 150 \times 4.6 5 μ m LiChrosorb RP-18

Mobile phase: MeOH:10 mM pH 7.5 Na₂HPO₄ 30:70

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: UV 228

CHROMATOGRAM

Retention time: 7

Internal standard: nizatidine

Limit of detection: 50 ng/mL

REFERENCE

Boom, S.P.A.; Moons, M.M.; Russel, F.G.M. Renal tubular transport of cimetidine in the isolated perfused kidney of the rat. *Drug Metab.Dispos.*, **1994**, *22*, 148-153

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

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-C₁ and Caco-2 cell lines as models for studying intestinal paracellular drug absorption. *Pharm.Res.*, **1996**, *13*, 216-221

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' 0.54

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, 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, tripeleppamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszán, R.; Nasal, A.; Turowski, M. Binding site for basic drugs on α₁-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: 5.47 (A), 3.16 (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, cispripide, 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, methyl dopa, 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, sulfipyrazone, 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

some 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 Partisil-10 ODS-3**Mobile phase:** MeOH:50 mM KH₂PO₄ 15:85, adjusted to pH 2.7**Flow rate:** 2**Injection volume:** 100**Detector:** UV 228

CHROMATOGRAM**Retention time:** 6-8**Internal standard:** codeine (10-12)

REFERENCE

Mummaneni, V.; Amidon, G.L.; Dressman, J.B. Gastric pH influences the appearance of double peaks in the plasma concentration-time profiles of cimetidine after oral administration in dogs. *Pharm.Res.*, 1995, 12, 780-786

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, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, 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, fenopropfen, 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, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylpyrrolone, meto-

prolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naprofen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrillamine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, 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, tripeleennamine, triproilidine, 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: Dilute solution 10-fold with mobile phase. Mix 100 μL with 100 μL 300 $\mu\text{g}/\text{mL}$ hydrocortisone, inject 10 μL .

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeCN:50 mM sodium acetate 33:67

Flow rate: 1

Injection volume: 10

Detector: UV 248

CHROMATOGRAM

Retention time: 7.1

Internal standard: hydrocortisone

KEY WORDS

water; stability-indicating

REFERENCE

Mahata, M.C.; Morosco, R.S.; Hipple, T.F. Stability of cimetidine hydrochloride and of clindamycin phosphate in water for injection stored in glass vials at two temperatures. *Am. J. Hosp. Pharm.*, 1993, 50, 2559-2561

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 $\mu\text{g}/\text{mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 \times 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: 1.22

OTHER SUBSTANCES

Simultaneous: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, 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, dipyrindamole, 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, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phenotolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pirtramide, 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, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, 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

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Busch, U.; Heinzl, G.; Narjes, H.; Nehmiz, G. Interaction of meloxicam with cimetidine, Maalox, or aspirin. *J.Clin.Pharmacol.*, **1996**, *36*, 79–84 [also aspirin, meloxicam+asma; pharmacokinetics]

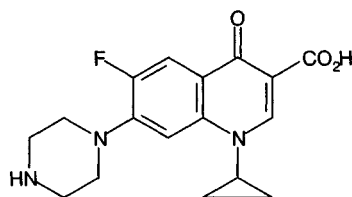
- Hermansson, J.; Grahn, A. Resolution of racemic drugs on a new chiral column based on silica-immobilized cellobiohydrolase. Characterization of the basic properties of the column. *J. Chromatogr.*, **1994**, *687*, 45–59 [chiral; also acebutolol, atenolol, betaxolol, bisoprolol, carbuterol, cathinone, dobutamine, dopropizine, epanolol, epinephrine, laudanosine, metanephrine, metoprolol, moprolool, norepinephrine, normetanephrine, octopamine, oxybutynine, pamatolol, practolol, prilocaine, propafenone, proxiphylline, sotalol, talinolol, tetrahydropapaveroline, tetramisole, timolol, tolamolol, toliprolol]
- Russel, F.G.; Creemers, M.C.; Tan, Y.; van Riel, P.L.; Gribnau, F.W. Ion-pair solid-phase extraction of cimetidine from plasma and subsequent analysis by high-performance liquid chromatography. *J. Chromatogr. B*, **1994**, *661*, 173–177 [plasma; serum; SPE; ranitidine (IS); LOD 5 ng/mL; LOQ 25 ng/mL; column temp 40]
- Kaka, J.S. Rapid method for cimetidine and ranitidine determination in human and rat plasma by HPLC. *J. Liq. Chromatogr.*, **1988**, *11*, 3447–3456 [LOD 50–100 ng/mL]
- Tracqui, A.; Kintz, P.; Mangin, P.; Lugnier, A.A.; Chaumont, A.J. A new rapid HPLC assay for the simultaneous determination of two histamine H₂-receptor antagonists, cimetidine and ranitidine, in human plasma. *J. Toxicol. Clin. Exp.*, **1988**, *8*, 387–394 [LOD 25 ng/mL; plasma]
- Wong, S.H.Y.; McHugh, S.L.; Dolan, J.; Cohen, K.A. Tricyclic antidepressant analysis by reversed-phase liquid chromatography using phenyl columns. *J. Liq. Chromatogr.*, **1986**, *9*, 2511–2538 [also acetaminophen, amitriptyline, amobarbital, amoxapine, barbital, chlordiazepoxide, chlorpromazine, clomipramine, codeine, desipramine, desmethyldoxepin, diazepam, doxepin, fluphenazine, flurazepam, glutethimide, hydroxyamoxapine, imipramine, lorazepam, maprotiline, meperidine, metabolites, nortriptyline, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, propoxyphene, protriptyline, secobarbital, thioridazine, trazodone]
- Wong, S.H.Y.; Marzouk, N.; McHugh, S.L.; Cazes, E. Simultaneous determination of theophylline and caffeine by reversed phase liquid chromatography using phenyl column. *J. Liq. Chromatogr.*, **1985**, *8*, 1797–1816 [also acetaminophen, caffeine, cimetidine, codeine, dimethylxanthine, meperidine, pentobarbital, phenobarbital, secobarbital, theobromine, theophylline; hydroxyethyltheophylline (IS)]
- Boutagy, J.; More, D.G.; Munro, I.A.; Shenfield, G.M. Simultaneous analysis of cimetidine and ranitidine in human plasma by HPLC. *J. Liq. Chromatogr.*, **1984**, *7*, 1651–1664 [also metabolites, N-acetylprocainamide, procainamide]
- Elliott, G.T.; McKenzie, M.W.; Curry, S.H.; Pieper, J.A.; Quinn, S.L. Stability of cimetidine hydrochloride in admixtures after microwave thawing. *Am. J. Hosp. Pharm.*, **1983**, *40*, 1002–1006 [stability-indicating; 5% dextrose; saline]
- Apffel, J.A.; Brinkman, U.A.T.; Frei, R.W. Analysis of cimetidine in biological fluids by high performance liquid chromatography. *J. Liq. Chromatogr.*, **1982**, *5*, 2413–2422 [urine; procaine (IS); blood; SPE]
- Fleitman, J.; Torosian, G.; Perrin, J.H. Improved high-performance liquid chromatographic assay for cimetidine using ranitidine as an internal standard. *J. Chromatogr.*, **1982**, *229*, 255–258 [plasma; ranitidine (IS); LOD 50 ng/mL]
- Mihaly, G.W.; Cockbain, S.; Jones, D.B.; Hanson, R.G.; Smallwood, R.A. High-pressure liquid chromatographic determination of cimetidine in plasma and urine. *J. Pharm. Sci.*, **1982**, *71*, 590–592 [plasma; urine; LOD 25 ng/mL; pharmacokinetics; procainamide can be used as IS (*J. Pharm. Sci.* 1984, *73*, 1015)]
- Kunitani, M.G.; Johnson, D.A.; Upton, R.A.; Riegelman, S. Convenient and sensitive high-performance liquid chromatography assay for cimetidine in plasma or urine. *J. Chromatogr.*, **1981**, *224*, 156–161 [plasma; urine; non-interfering acebutolol, caffeine, ketoprofen, naproxen, theophylline; pharmacokinetics; LOQ 100 ng/mL]
- Lorenzo, B.; Drayer, D.E. Improved method for the measurement of cimetidine in human serum by reverse-phase high-pressure liquid chromatography. *J. Lab. Clin. Med.*, **1981**, *97*, 545–550 [serum; plasma; LOD 50 ng/mL]
- Ziemniak, J.A.; Chiaromonte, D.A.; Schentag, J.J. Liquid-chromatographic determination of cimetidine, its known metabolites, and creatinine in serum and urine. *Clin. Chem.*, **1981**, *27*, 272–275 [serum; plasma; urine; extracted metabolites, creatinine; pharmacokinetics; LOD 50 ng/mL]
- La Rotonda, M.I.; Cozzolino, S.; Schettino, O. [Analysis of active principles in pharmaceutical dosage forms by high pressure liquid chromatography—cimetidine and zolimidine]. *Boll. Soc. Ital. Biol. Sper.*, **1980**, *56*, 1394–1398

Ciprofloxacin

Molecular formula: C₁₇H₁₈FN₃O₃

Molecular weight: 331.4

CAS Registry No.: 85721-33-1, 86393-32-0
(hydrochloride monohydrate)



SAMPLE

Matrix: bile, perfusate

Sample preparation: Add an equal volume of MeCN to perfusate or bile, centrifuge, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 5 μ m RP-18 (Merck)

Mobile phase: MeCN:buffer 22:78 (Buffer was 25 mM pH 3 phosphate containing 5 mM tetraethylammonium bromide.)

Flow rate: 1.5

Injection volume: 50

Detector: F ex 270 em 440

CHROMATOGRAM

Retention time: 3.5

Limit of detection: 100 ng/mL

KEY WORDS

rat; liver; pharmacokinetics

REFERENCE

Abadia, A.R.; De Francesco, L.; Guaitani, A. Disposition of ciprofloxacin in the isolated perfused rat liver. *Drug Metab. Dispos.*, **1995**, *23*, 197-200

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 50 μ L 20 μ g/mL pipemidic acid + 200 μ L 25% sodium sulfate + 3.5 mL dichloromethane, extract. Extract the organic phase with 200 μ L 100 mM NaOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: C18

Mobile phase: MeCN:buffer 15:85 (Buffer was 10 mM NaH₂PO₄ and 5 mM tetrabutylammonium hydrogen sulfate, pH 2.7.)

Flow rate: 2

Injection volume: 20

Detector: F ex 278

CHROMATOGRAM

Internal standard: pipemidic acid

Limit of quantitation: 100 ng/mL

KEY WORDS

serum; pharmacokinetics

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

Sample preparation: Precipitate proteins with MeCN and perchloric acid, inject an aliquot.

HPLC VARIABLES

Guard column: Alltech C18

Column: 150 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:100 mM citric acid 4:21:75 containing 0.54 g/L ammonium perchlorate and 0.65 mL/L tetrabutylammonium hydroxide

Flow rate: 1

Detector: F ex 270 em 440

CHROMATOGRAM

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Shah, A.; Lettieri, J.; Nix, D.; Wilton, J.; Heller, A.H. Pharmacokinetics of high-dose intravenous ciprofloxacin in young and elderly and in male and female subjects. *Antimicrob. Agents Chemother.*, **1995**, *39*, 1003–1006

SAMPLE

Matrix: blood

Sample preparation: 100 μL Plasma + 100 μL pH 7.4 phosphate buffer + 50 μL 20 μg/mL β-hydroxypropyltheophylline in pH 7.4 phosphate buffer + 5 mL chloroform:isopropanol 95:5, shake on a rotary mixer for 15 min, centrifuge at 800 g for 5 min. Evaporate organic layer under nitrogen at 45°, sonicate residue with 100 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.9 Spherisorb ODS

Column: 250 × 4.9 Spherisorb S5 ODS2

Mobile phase: MeCN:buffer 15:85 adjusted, to pH 3.0 with 85% phosphoric acid immediately before use. (Buffer was 4.54 g KH₂PO₄ + 5.94 g Na₂HPO₄·2H₂O + 1.49 g tetrabutylammonium hydrogen sulfate per L.)

Flow rate: 1.3

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 6.2

Internal standard: β-hydroxypropyltheophylline

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: enoxacin, norfloxacin, theophylline

KEY WORDS

plasma; rat

REFERENCE

Davis, J.D.; Aarons, L.; Houston, J.B. Simultaneous assay of fluoroquinolones and theophylline in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, 621, 105–109

SAMPLE**Matrix:** blood

Sample preparation: 200 μ L Serum + 50 μ L 40 μ g/mL quinine hemisulfate in water, vortex 30 s, add 400 μ L MeCN, vortex 1 min, centrifuge at 4000 rpm for 10 min. Remove organic layer and evaporate to 200 μ L under a stream of dry nitrogen at 45°. Inject 10–20 μ L aliquot.

HPLC VARIABLES**Column:** 10 μ m μ Bondapak C18**Mobile phase:** MeCN:100 mM NaH₂PO₄ adjusted to pH 3.9 with phosphoric acid 20:80**Flow rate:** 2.5**Injection volume:** 10–20**Detector:** F ex 280 em 455

CHROMATOGRAM**Retention time:** 4.0**Internal standard:** quinine**Limit of detection:** 25 ng/mL

OTHER SUBSTANCES**Simultaneous:** acebutolol, lomefloxacin, norfloxacin, ofloxacin, pefloxacin

Noninterfering: atenolol, deoxyphenaline, digoxin, gentamicin, hyoscine, metoclopramide, metronidazole, midodrine, nadolol, netilmicin, prednisolone, ranitidine, verapamil, vitamin B1

KEY WORDS

serum

REFERENCE

Jim, L.K.; el-Sayed, N.; al-Khamis, K.I. A simple high-performance liquid chromatographic assay for ciprofloxacin in human serum. *J.Clin.Pharm.Ther.*, **1992**, 17, 111–115

SAMPLE**Matrix:** blood

Sample preparation: 50 μ L Plasma + 1 mL 100 mM pH 7.0 K₂HPO₄ adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μ L 300 μ g/mL nalidixic acid in water + 3 mL dichloromethane:isoamyl alcohol 9:1, shake vigorously for 10 min, centrifuge at 2270 g for 10 min. Remove 2 mL of the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute residue in 100 μ L MeOH:50 mM NaOH 2:1, vortex, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Chemcosorb 5-ODS-H**Mobile phase:** MeOH:5 mM sodium lauryl sulfate 2:1, adjusted to pH 2.35 with 85% phosphoric acid**Column temperature:** 40**Flow rate:** 0.6

Injection volume: 10

Detector: UV 275

CHROMATOGRAM

Retention time: 6

Internal standard: nalidixic acid (5)

Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Extracted: felbinac, fenbufen

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Naora, K.; Katagiri, Y.; Ichikawa, N.; Hayashibara, M.; Iwamoto, K. Simultaneous high-performance liquid chromatographic determination of ciprofloxacin, fenbufen and felbinac in rat plasma. *J.Chromatogr.*, **1990**, *530*, 186–191

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 500 μ L 7% perchloric acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 3.9 Nova-Pak C18

Mobile phase: MeOH:18 mM KH₂PO₄ containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1

Injection volume: 20

Detector: F ex 278 em 475

CHROMATOGRAM

Retention time: 8.5

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Griggs, D.J.; Wise, R. A simple isocratic high-pressure liquid chromatographic assay of quinolones in serum. *J.Antimicrob.Chemother.*, **1989**, *24*, 437–445

SAMPLE

Matrix: blood, intestinal efflux

Sample preparation: Intestinal efflux. Freeze intestinal efflux at -80°, lyophilize, reconstitute with 1 mL ofloxacin in MeOH:100 mM phosphoric acid 50:50, centrifuge at 3000 rpm for 10 min, inject a 20 μ L aliquot. Serum. Deproteinize serum with MeOH containing ofloxacin.

HPLC VARIABLES

Column: 150 \times 3.9 Novapack C18

Mobile phase: MeOH:buffer 25:75 (Buffer was 10 mM pH 3.0 potassium phosphate buffer containing 25 mM sodium heptanesulfonate (PIC B7) and 20 mM triethylamine.)

Flow rate: 1.5

Injection volume: 20

Detector: F ex 330 em 440

CHROMATOGRAM

Internal standard: ofloxacin

Limit of detection: 10 ng/mL

KEY WORDS

serum; rat

REFERENCE

Rubinstein, E.; Dautrey, S.; Farinoti, R.; St.Julien, L.; Ramon, J.; Carbon, C. Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats. *Antimicrob.Agents Chemother.*, **1995**, *39*, 99–102

SAMPLE

Matrix: blood, middle-ear fluid

Sample preparation: 50 μ L Plasma or middle-ear fluid + 20 μ L 10 μ g/mL difloxacin in water + 2 mL MeCN, vortex, centrifuge at 1500 g for 10 min. Evaporate organic layer to dryness under nitrogen at 50°, reconstitute in 75 μ L mobile phase, inject 5 μ L.

HPLC VARIABLES

Guard column: 10 \times 2.1 5 μ m C18 Hypersil

Column: 100 \times 2.1 5 μ m C18 Hypersil

Mobile phase: MeCN:buffer 40:60 (Buffer was 30 mM NaH₂PO₄, 20 mM triethylamine, 20 mM sodium dodecyl sulfate adjusted to pH 3.0 with phosphoric acid.)

Column temperature: 45

Flow rate: 0.35

Injection volume: 5

Detector: F ex 278 em 456

CHROMATOGRAM

Retention time: 3.0

Internal standard: difloxacin

Limit of detection: 5 ng/mL

KEY WORDS

plasma; chinchilla

REFERENCE

Lovdahl, M.; Steury, J.; Russlie, H.; Canafax, D.M. Determination of ciprofloxacin levels in chinchilla middle ear effusion and plasma by high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1993**, *617*, 329–333

SAMPLE

Matrix: blood, milk

Sample preparation: 500 μ L Milk or plasma + 500 μ L MeCN:100 mM NaOH, vortex for 10-15 s, filter (Centricon-3, 3000 Dalton cut-off) while centrifuging at 4000 g for 30 min, inject a 50-150 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 \times 4.6 3 μ m Spherisorb phenyl

Mobile phase: MeCN:MeOH:triethylamine:85% phosphoric acid:water 9:9:0.45:0.4:81.15 containing 5 mM dodecanesulfonate

Column temperature: 50
Flow rate: 1
Injection volume: 50-150
Detector: UV 278

CHROMATOGRAM

Retention time: 10.7
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: enrofloxacin

KEY WORDS

plasma; cow; ultrafiltrate

REFERENCE

Tyczkowska, K.L.; Voyksner, R.D.; Anderson, K.L.; Papich, M.G. Simultaneous determination of enrofloxacin and its primary metabolite ciprofloxacin in bovine milk and plasma by ion-pairing liquid chromatography. *J.Chromatogr.B*, **1994**, 658, 341-348

SAMPLE

Matrix: blood, saliva

Sample preparation: 500 μ L Plasma or saliva + 50 μ L 50 ng/mL difloxacin, vortex briefly, add 500 μ L 100 mM pH 7.4 phosphate buffer, add 4 mL dichloromethane, add 1 mL isopropanol, vortex for 30 s, shake gently for 30 min, centrifuge at 1500 g for 20 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 μ L mobile phase, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 Guard-Pak

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:buffer 35:100 (Buffer was 5.44 g KH_2PO_4 and 4 mL tetrabutylammonium hydroxide in 1 L water, adjust pH to 2.5 with 85% phosphoric acid.)

Flow rate: 2

Injection volume: 50-200

Detector: UV 268

CHROMATOGRAM

Retention time: 7.1

Internal standard: difloxacin (8.8)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: enoxacin, theophylline

Simultaneous: caffeine, 1,7-dimethylxanthine

Noninterfering: 1,3-dimethyluric acid, hypoxanthine, 1-methyluric acid, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, theobromine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Zhai, S.; Korrapati, M.R.; Wei, X.; Muppalla, S.; Vestal, R.E. Simultaneous determination of theophylline, enoxacin and ciprofloxacin in human plasma and saliva by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 669, 372-376

SAMPLE

Matrix: blood, tissue

Sample preparation: Lung. Homogenize (Ultra-Turrax T25) mouse lung in 1-3 mL pH 6.8 Soerensen phosphate buffer, centrifuge. Mix an aliquot of the supernatant with an equal volume of MeCN, centrifuge, inject a 5-20 μ L aliquot of the supernatant. Serum. Mix serum with an equal volume of MeCN, centrifuge, inject a 5-20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrabase C8 (SFCC, Neuilly Plaisance, France)

Mobile phase: MeCN:MeOH:5% acetic acid 5:3:92

Flow rate: 1

Injection volume: 5-20

Detector: F ex 278 em 418

CHROMATOGRAM

Retention time: 5

Limit of detection: 15 ng

KEY WORDS

serum; lung; mouse; pharmacokinetics

REFERENCE

Vallée, E.; Azoulay-Dupuis, E.; Bauchet, J.; Pocardalo, J.-J. Kinetic disposition of temafloxacin and ciprofloxacin in a murine model of pneumococcal pneumonia. Relevance for drug efficacy. *J.Pharmacol.Exp.Ther.*, **1992**, *262*, 1203-1208

SAMPLE

Matrix: blood, tissue

Sample preparation: Serum. 500 μ L Serum + 500 μ L MeCN:100 mM NaOH 50:50, vortex for 10-15 s, filter (Amicon Centricon-10, 10000 Daltons) while centrifuging at 2677 g for 30 min, inject a 30-120 μ L aliquot of the ultrafiltrate. Tissue. Cut up prostate tissue with a scalpel. Weigh out 100-130 mg tissue, make up to 500 μ L with MeCN:100 mM NaOH 50:50, sonicate for 30 min, filter (Amicon Centricon-10, 10000 Daltons) while centrifuging at 2677 g for 30 min, inject a 80-120 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Spherisorb phenyl

Mobile phase: MeCN:MeOH:water 15:2:83 containing 3 mM dodecanesulfonate, 1.5 mM octanesulfonate, 0.4% phosphoric acid, and 0.4% triethylamine

Column temperature: 40

Injection volume: 30-120

Detector: UV 278.6

CHROMATOGRAM

Retention time: 7.75

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: enrofloxacin

KEY WORDS

serum; dog; prostate; ultrafiltrate

REFERENCE

Tyczkowska, K.; Hedeem, K.M.; Aucoin, D.P.; Aronson, A.L. High-performance liquid chromatographic method for the simultaneous determination of enrofloxacin and its primary metabolite ciprofloxacin in canine serum and prostatic tissue. *J.Chromatogr.*, **1989**, *493*, 337–346

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with 30 mM phosphoric acid, centrifuge, inject a 20 μ L aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with 30 mM phosphoric acid, centrifuge, inject a 20 μ L 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 μ L 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 μ L aliquot. Pleural. Dilute human pleural samples with buffer, centrifuge, inject a 20 μ L aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4 .)

HPLC VARIABLES

Column: 200 \times 4.5 μ m Nucleosil C18

Mobile phase: MeOH:MeCN:buffer 13:7:80, adjusted to pH 3.0 with phosphoric acid (Buffer was 15 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide.)

Flow rate: 1

Injection volume: 20-100

Detector: F ex 278 em 446

CHROMATOGRAM

Retention time: 6

Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: norfloxacin, ofloxacin

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: blood, urine

Sample preparation: Blood. 500 μ L Serum or plasma + 100 μ L 20 μ g/mL IS in 100 mM phosphoric acid + 300 μ L MeCN:5 M trichloroacetic acid 50:50, vortex, add 100 μ L MeCN, add 300 μ L water, vortex, centrifuge at 1500 g for 15 min, inject a 10 μ L aliquot of the supernatant. Urine. Dilute urine 1:20 (or more) with 50 mM pH 3.0 KH_2PO_4 , remove a 500 μ L aliquot and add it to 100 μ L 20 μ g/mL IS in 100 mM phosphoric acid, add 700 μ L 100 mM trichloroacetic acid, vortex, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 5 \times 3 PLRP-S (Polymer Laboratories)

Column: 150 \times 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

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: Extract with chloroform:isopropanol 95:5.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeOH:40 mM potassium phosphate + 20 mM sodium phosphate 65:35, containing 2 g/L hexadecyltrimethylammonium bromide, pH adjusted to 7.4 with HCl

Flow rate: 1

Detector: F ex 280 em 425

CHROMATOGRAM

Retention time: 4.5

Internal standard: feroxacin

Limit of detection: 30 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: blood, urine

Sample preparation: Dilute with one or more volumes of water, filter (0.6 μ m)

HPLC VARIABLES

Column: 200 × 4.5 μ m Nucleosil C18

Mobile phase: MeCN:25 mM orthophosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 11:89

Flow rate: 1.5

Injection volume: 10-20

Detector: F ex 278 em 445

CHROMATOGRAM**Retention time:** 3.1**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Noninterfering:** acetaminophen, cefuroxime, cloxacillin, dextropropoxyphene, digoxin, doxycycline, erythromycin, furosemide, metronidazole, netilmicin, penicillin G, prednisolone, salicylic acid, sulfamethoxazole, trimethoprim, warfarin**Interfering:** norfloxacin

KEY WORDS

serum

REFERENCENilsson-Ehle, I. Assay of ciprofloxacin and norfloxacin in serum and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *416*, 207–211

SAMPLE**Matrix:** blood, urine**Sample preparation:** Serum. 300 μ L Serum + 300 μ L 6% aqueous trichloroacetic acid, centrifuge at 5000 rpm for 5 min, inject 10 μ L of supernatant. Urine. Dilute 1:1000 or 1:100 with mobile phase, inject 10 μ L directly.

HPLC VARIABLES**Column:** 250 \times 4.6 Spherisorb ODS**Mobile phase:** MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 11:89**Flow rate:** 2**Injection volume:** 10**Detector:** F ex 278 em 456

CHROMATOGRAM**Retention time:** 4

KEY WORDSserum; a similar analysis is stability-indicating (*Am.J.Hosp.Pharm.* 1994, *51*, 373-7)

REFERENCEJoos, B.; Ledergerber, B.; Flepp, M.; Bettex, J.-D.; Lüthy, R.; Siegenthaler, W. Comparison of high-pressure liquid chromatography and bioassay for determination of ciprofloxacin in serum and urine. *Antimicrob.Agents Chemother.*, **1985**, *27*, 353–356

SAMPLE**Matrix:** blood, vitreous humor**Sample preparation:** Serum. 20 μ L Serum + 130 μ L mobile phase, mix, filter, inject a 100 μ L aliquot. Vitreous humor. 15 μ L Vitreous humor + 135 μ L mobile phase, mix, filter, inject a 100 μ L aliquot.

HPLC VARIABLES**Column:** 220 \times 2.1 5 μ m Nucleosil C18**Mobile phase:** MeCN:MeOH:50 mM KH_2PO_4 :100 mM tetrabutylammonium hydroxide 10:7:73:10**Column temperature:** 25**Flow rate:** 0.2

Injection volume: 100

Detector: UV 240; UV 280; F ex 280 em 445

CHROMATOGRAM

Limit of detection: 2 ng/mL

KEY WORDS

serum; rabbit; pharmacokinetics

REFERENCE

Drusano, G.L.; Liu, W.; Perkins, R.; Madu, A.; Madu, C.; Mayers, M.; Miller, M.H. Determination of robust ocular pharmacokinetic parameters in serum and vitreous humor of albino rabbits following systemic administration of ciprofloxacin from sparse data sets by using IT2S, a population pharmacokinetic modeling program. *Antimicrob.Agents Chemother.*, **1995**, *39*, 1683–1687

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 100 µg/mL solution in mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.5 10 µm C18 (Flexit, Pune, India)

Mobile phase: MeOH: water: acetic acid 84:15.9:0.1

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 2.25

Limit of detection: 5 ng

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Husain, S.; Khalid, S.; Nagaraju, V.; Rao, R.N. High-performance liquid chromatographic separation and determination of small amounts of process impurities of ciprofloxacin in bulk drugs and formulations. *J.Chromatogr.A*, **1995**, *705*, 380–384

SAMPLE

Matrix: cell suspensions

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 the supernatant under a stream of air, dissolve the residue in 100 µL mobile phase, inject a 75 µL aliquot.

HPLC VARIABLES

Column: µBondapak C18

Mobile phase: MeCN: 5 mM pH 2.0 tetrabutylammonium hydrogen sulfate 10:90

Flow rate: 1

Injection volume: 75

Detector: UV 280

CHROMATOGRAM

Retention time: 14

Internal standard: ofloxacin

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: cells

Sample preparation: Incubate cells in 2 mL 100 mM pH 3.0 glycine-HCl buffer for 2 h at room temperature, centrifuge at 5600 g for 5 min, inject an aliquot.

HPLC VARIABLES

Column: Bondapak C18

Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 25:75

Flow rate: 1.5

Detector: F ex 340 em 425

OTHER SUBSTANCES

Also analyzed: fleroxacin, lomefloxacin, norfloxacin, ofloxacin, temafloxacin

REFERENCE

Pascual, A.; Garcia, I.; Conejo, M.C.; Perea, E.J. Fluorometric and high-performance liquid chromatographic measurement of quinolone uptake by human neutrophils. *Eur.J.Clin.Microbiol.Infect.Dis.*, **1991**, *10*, 969-971

SAMPLE

Matrix: hair

Sample preparation: Wash hair successively with 0.1% sodium dodecyl sulfate and water for 30 min, repeat twice, blot between 2 sheets of paper towel, allow to dry at room temperature. Take a 1 cm fragment of hair, add 500 μ L 1 M NaOH, heat at 80° for 30 min, cool, add 500 μ L 1 M HCl, add 1 mL 100 mM pH 4.6 potassium hydrogen citrate buffer, add 50 μ L 1 μ g/mL IS in water. Add the mixture to a Bond-Elut C8 SPE cartridge, elute with 2 mL THF:25 mM orthophosphoric acid 20:80, evaporate eluate to dryness in vacuum, dissolve residue in 150 μ L mobile phase, vortex, inject a 60 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 Tosoh 5 μ m TSKgel ODS-80Ts

Mobile phase: MeCN:25 mM orthophosphoric acid adjusted to pH 3.0 with 0.5 M tetrabutylammonium hydroxide 5:95

Column temperature: 40

Flow rate: 1

Injection volume: 60

Detector: F ex 280 em 445

CHROMATOGRAM

Retention time: 13.5

Internal standard: (R)-9-fluoro-2,3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (DS-4632) (10.2)

Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES

Simultaneous: norfloxacin, ofloxacin (F ex 295 em 490)

KEY WORDS

SPE

REFERENCE

Mizuno, A.; Uematsu, T.; Nakashima, M. Simultaneous determination of ofloxacin, norfloxacin and ciprofloxacin in human hair by high-performance liquid chromatography and fluorescence detection. *J.Chromatogr.B*, **1994**, *653*, 187–193

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 \times 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:** solutions**Sample preparation:** Prepare a 20 $\mu\text{g}/\text{mL}$ solution in MeCN:water 10:90, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4.5 μm LiChrospher 100 RP-18**Mobile phase:** MeCN:25 mM phosphoric acid 7:93, adjusted to pH 3.09 with 100 mM tetrabutylammonium hydroxide**Flow rate:** 1**Injection volume:** 10**Detector:** UV 280

CHROMATOGRAM**Retention time:** 9.8

OTHER SUBSTANCES**Simultaneous:** norfloxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE

Barbosa, J.; Bergés, R.; Sanz-Nebot, V. Linear solvation energy relationships in reversed-phase liquid chromatography. Prediction of retention of several quinolones. *J.Liq.Chromatogr.*, **1995**, *18*, 3445–3463

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: MeCN:20 mM pH 2.3 phosphoric acid 15:85 containing 2.5 mM sodium 1-heptanesulfonate

Flow rate: 1.5

Detector: UV 278

CHROMATOGRAM

Retention time: 25

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Torniainen, K.; Mäki, E. Development of an isocratic high-performance liquid chromatographic method for monitoring of ciprofloxacin photodegradation. *J.Chromatogr.A*, **1995**, 697, 397–405

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 277

CHROMATOGRAM

Retention time: k' 3.1

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

HPLC VARIABLES

Column: 150 × 4.6 5 μm BDS-Hypersil C18

Mobile phase: MeOH:THF:670 mM pH 3.0 phosphate buffer 20:0.8:79.2 plus 2 g/L tetrabutylammonium hydrogen sulfate and 2 mL/L 85% phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 275

CHROMATOGRAM

Retention time: 5.83

OTHER SUBSTANCES**Simultaneous:** photodegradation products**Interfering:** fleroxacin, ofloxacin

KEY WORDS

water

REFERENCE

Tiefenbacher, E.-M.; Haen, E.; Przybilla, B.; Kurz, H. Photodegradation of some quinolones used as antimicrobial therapeutics. *J.Pharm.Sci.*, **1994**, *83*, 463–467

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 450 µg/mL solution in MeCN:water 50:50. 5 mL Solution + 5 mL THF + 200 molar excess of acetic anhydride + 3 molar excess of 1 M NaOH, sonicate for 15 min, add 15 mL mobile phase, sonicate for 15 min, cool to room temperature, make up to 50 mL with mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 5 µm Nucleosil C18**Mobile phase:** MeCN:buffer 35:65 (Buffer was prepared by mixing equal volumes of 20 mM citric acid and 20 mM sodium citrate, pH adjusted to 2.4 with perchloric acid.)**Flow rate:** 1**Injection volume:** 50**Detector:** UV 280

CHROMATOGRAM**Retention time:** 6.4

OTHER SUBSTANCES**Simultaneous:** norfloxacin, sarafloxacin, temafloxacin

KEY WORDS

derivatization

REFERENCE

Morley, J.A.; Elrod, L., Jr. Determination of fluoroquinolone antibacterials as N-Acyl derivatives. *Chromatographia*, **1993**, *37*, 295–299

SAMPLE**Matrix:** tissue**Sample preparation:** Wash a 500 mg 2.8 mL Bond-Elut SCX cartridge with 1% acetic acid in EtOH. Homogenize 2 g muscle tissue in 20 mL 1% acetic acid in EtOH, sonicate 3 min, centrifuge at 4200 g for 5 min, decant supernatant. Repeat extraction, combine supernatants, centrifuge at 4200 g for 5 min. Pass supernatants through SPE cartridge, wash cartridge with 5 mL MeOH, 10 mL water, 5 mL MeOH, and elute with 25% aqueous ammonia (specific gravity 0.88) in MeOH. Evaporate eluate to dryness under a stream of nitrogen at 50°, evaporation of the final portion is aided by the addition of 1 mL MeCN. Add 1 mL mobile phase, vortex 15 s, sonicate 3 min, centrifuge at 1860 g for 5 min, filter (0.45 µm), inject 20 µL.

HPLC VARIABLES**Column:** 250 × 4.6 Zorbax RXC8**Mobile phase:** MeCN:buffer 20:80 (Buffer was 0.68 M orthophosphoric acid in 900 mL water, taken to pH 3.0 with triethylamine, made up to 1 L.)

Flow rate: 0.5
Injection volume: 20
Detector: F ex 278 em 445

CHROMATOGRAM

Retention time: 10
Limit of quantitation: <10 ng/g

OTHER SUBSTANCES

Simultaneous: enrofloxacin

KEY WORDS

muscle; pig; cow; SPE

REFERENCE

Tarbin, J.A.; Tyler, D.J.; Shearer, G. Analysis of enrofloxacin and its metabolite ciprofloxacin in bovine and porcine muscle by high-performance liquid chromatography following cation exchange clean-up. *Food Addit. Contam.*, **1992**, 9, 345–350

SAMPLE

Matrix: urine
Sample preparation: 50 μ L Urine + 100 μ L IS + 3.85 mL water, mix, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Nucleosil C18
Mobile phase: MeCN:50 mM citric acid:1 M ammonium acetate 22:77:1
Flow rate: 1.5
Injection volume: 10
Detector: F ex 280 em 418

CHROMATOGRAM

Retention time: 3.8
Internal standard: KK-123 (G.D.Searle) (6.3)
Limit of quantitation: 2 μ g/mL

OTHER SUBSTANCES

Extracted: lomefloxacin

KEY WORDS

plasma

REFERENCE

Stuht, H.; Lode, H.; Koeppe, P.; Rost, K.L.; Schaberg, T. Interaction study of lomefloxacin and ciprofloxacin with omeprazole and comparative pharmacokinetics. *Antimicrob. Agents Chemother.*, **1995**, 39, 1045–1049

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Delon, A.; Favreliere, S.; Couet, W.; Courtois, P.; Bouquet, S. Rapid and sensitive determination of thalidomide in human plasma by high-performance liquid chromatography. *J. Liq. Chromatogr.*, **1995**, 18, 297–309 [SPE; ciprofloxacin is IS; simultaneous acyclovir, azathioprine, cefotaxime, ceftazidime, flucytosine, metronidazole; non-interfering amphotericin, clobazam, clonazepam, cyclophosphamide, cyclosporin, diazepam, diltiazem, hydroxyzine, nifedipine, prednisolone]

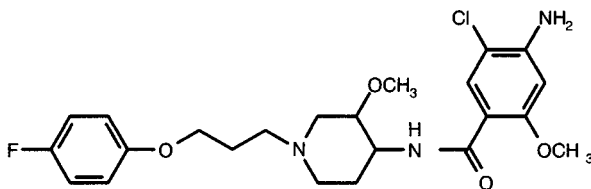
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- Davis, J.D.; Aarons, L.; Houston, J.B. Relationship between enoxacin and ciprofloxacin plasma concentrations and theophylline disposition. *Pharm.Res.*, **1994**, *11*, 1424–1428 [extracted enoxacin, theophylline; plasma; hydroxypropyltheophylline (IS); LOD 500 ng/mL]
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- Gau, W.; Ploschke, H.J.; Schmidt, K.; Weber, B. Determination of ciprofloxacin (BAY o 9867) in biological fluids by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1985**, *8*, 485–497 [serum; plasma; urine; fluorescence detection; LOD 8 ng/mL (plasma, serum); LOD 50 ng/mL (urine); pharmacokinetics]
- Aronoff, G.E.; Kenner, C.H.; Sloan, R.S.; Pottratz, S.T. Multiple-dose ciprofloxacin kinetics in normal subjects. *Clin.Pharmacol.Ther.*, **1984**, *36*, 384–388 [plasma; urine; fluorescence detection; column temp 58; LOD 30 ng/mL; pharmacokinetics]

Cisapride

Molecular formula: C₂₃H₂₉ClFN₃O₄

Molecular weight: 466.0

CAS Registry No.: 81098-60-4



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 70 μ L 1 M NaOH + 1 mL 30 ng/mL IS in chloroform:isopropanol 90:10, vortex for 2 min, centrifuge at 1800-1900 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 octyl Symmetry (Waters)

Mobile phase: MeCN:20 mM pH 5.2 phosphate buffer 37:63

Flow rate: 1

Detector: F ex 295 em 350

CHROMATOGRAM

Retention time: 5

Internal standard: cis-4-amino-5-chloro-N-[1-[5-(4-fluorophenoxy)pentyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide monohydrate (R 54 680, Jansen-Cilag) (8)

Limit of detection: 5 ng/mL

Limit of quantitation: 8 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, norcisapride

Simultaneous: furosemide

Noninterfering: amoxicillin, caffeine, dexamethasone, gentamicin, hydrocortisone, indomethacin, metoclopramide, midazolam, theobromine, theophylline, tolazoline

KEY WORDS

plasma

REFERENCE

Preechagoon, Y.; Charles, B.G. Analysis of cisapride in neonatal plasma using high-performance liquid chromatography with a base-stable column and fluorescence detection. *J.Chromatogr.B*, **1995**, 670, 139-143

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 2 mL Plasma + 100 ng IS + 500 μ L 1 M NaOH, mix, add 6 mL heptane:isoamyl alcohol 95:5, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 5 min. Remove the organic layer and add it to 3 mL 50 mM sulfuric acid, extract, centrifuge. Remove the aqueous layer and make it alkaline with 150 μ L concentrated ammonia, add 4 mL heptane:isoamyl alcohol 95:5, extract, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 120 μ L mobile phase, inject a 40 μ L aliquot. Tissue. Grind tissue (Waring blender), homogenize (Ultra-Turrax) with three volumes 10 mM pH 7.4 phosphate buffer containing 1.15% KCl. 1 mL Homogenate + 200 ng IS + 500 μ L 1 M NaOH, mix, add 6 mL heptane:isoamyl alcohol 95:5, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 5 min. Remove the organic layer and add it to 3 mL 50 mM sulfuric acid, extract, centrifuge. Remove the aqueous layer and make it alkaline with 150 μ L concentrated am-

monia, add 4 mL heptane:isoamyl alcohol 95:5, extract, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 120 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 2.1 5 μ m ODS-Hypersil

Mobile phase: MeCN:water:diethylamine 44:56:0.02

Flow rate: 0.8

Injection volume: 40

Detector: UV 276

CHROMATOGRAM

Retention time: 3.08

Internal standard: cis-4-amino-5-chloro-N-[1-[5-(4-fluorophenoxy)pentyl]-3-methoxy-4-piperidiny]-2-methoxybenzamide monohydrate (R 54 680) (5.96)

Limit of detection: 1 ng/mL

KEY WORDS

plasma; method can also be used for urine (*Drug Metab. Dispos.* 1988, 16, 403); method can also be used for human breast milk (*Eur.J.Clin.Pharmacol.* 1986, 30, 735); human; rat; liver

REFERENCE

Woestenborghs, R.; Lorreyne, W.; Van Rompaey, F.; Heykants, J. Determination of cisapride in plasma and animal tissues by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *424*, 195–200

SAMPLE

Matrix: feces, urine

Sample preparation: Urine. Inject a 275 μ L aliquot of urine directly. Feces. Extract feces with MeOH.

HPLC VARIABLES

Column: 300 \times 4.6 5 μ m Lichrosorb RP-8

Mobile phase: Gradient. A was 100 mM ammonium acetate containing 40 mM diisopropylamine, adjusted to pH 8.0 with ammonia. B was MeCN:MeOH:1 M pH 8.0 ammonium acetate containing 400 mM diisopropylamine 45:45:10. A:B from 90:10 to 30:70 over 40 min.

Flow rate: 1

Injection volume: 275

Detector: Radioactivity; UV 230; UV 306

CHROMATOGRAM

Retention time: 52

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; tritium labeled

REFERENCE

Meuldermans, W.; Hendrickx, J.; Lauwers, W.; Hurkmans, R.; Mostmans, E.; Swysen, E.; Bracke, J.; Knaeps, A.; Heykants, J. Excretion and biotransformation of cisapride in rats after oral administration. *Drug Metab.Dispos.*, **1988**, *16*, 410–419

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute syrup 10-fold with MeOH, remove a 200 μ L aliquot and add it to 20 μ L IS solution, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 160 \times 4.5 μ m Zorbax Rx-C8**Mobile phase:** MeCN:water:triethylamine 65:35:0.02**Flow rate:** 1**Injection volume:** 10**Detector:** UV 276

CHROMATOGRAM**Retention time:** 2.7**Internal standard:** cis-4-amino-5-chloro-n-{1-[5-(4-fluorophenoxy)pentyl]-3-methoxy-4-piperidiny]-2-methoxybenzamide monohydrate (3.8)

KEY WORDS

syrup; stability-indicating

REFERENCENahata, M.C.; Morosco, R.S.; Hipple, T.F. Stability of cisapride in a liquid dosage form at two temperatures. *Ann.Pharmacother.*, **1995**, *29*, 125–126

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 4.6 μ m Supelcosil LC-DP (A) or 250 \times 4.5 μ m 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:** 10.90 (A), 5.81 (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, 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, flvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydroxalazine, 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, sulfipyrazone, 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

some details of plasma extraction

REFERENCE

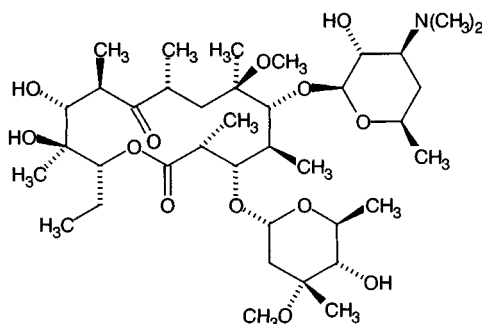
Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology. *J.Chromatogr.A*, **1995**, 692, 103–119

Clarithromycin

Molecular formula: C₃₈H₆₉NO₁₃

Molecular weight: 748.0

CAS Registry No.: 81103-11-9



SAMPLE

Matrix: alveolar cells, blood, bronchoalveolar lavage fluid

Sample preparation: Freeze dry bronchoalveolar lavage fluid and reconstitute in water to yield a 10-fold concentration. Suspend alveolar cells in pH 8.0 potassium phosphate buffer, sonicate (Fisher Model 50 Sonic Dismembrator) at 50% cycle for 2 min. 500 μ L Alveolar cell suspension, bronchoalveolar lavage fluid concentrate, or plasma 60 μ L 10 μ g/mL IS + 200 μ L 100 mM sodium carbonate + 3 mL hexane:ethyl acetate 50:50, vortex, centrifuge at 800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μ L MeCN:water 50:50, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 8 4 μ m Nova-Pak phenyl radial compression

Mobile phase: MeCN:MeOH:1 M NaH₂PO₄:water 35:4:4:57, pH 6.85

Flow rate: 1.6

Injection volume: 50

Detector: E, Environmental Sciences Associates Model 5100A, screen electrode +0.5 V, analytical cell +0.79 V, Model 5020 pre-column guard cell +0.85 V

CHROMATOGRAM

Internal standard: erythromycin A 9-O-methyloxime

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Conte, J.E., Jr.; Golden, J.A.; Duncan, S.; McKenna, E.; Zurlinden, E. Intrapulmonary pharmacokinetics of clarithromycin and of erythromycin. *Antimicrob. Agents Chemother.*, **1995**, *39*, 334-338

SAMPLE

Matrix: blood

Sample preparation: Condition a 10 \times 2 20 mg 30-40 μ m Baker CN SPE cartridge with 2 mL MeOH, 2 mL MeOH:water 10:90, and 4 mL MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90 at 2 mL/min. Centrifuge plasma at 1300 g for 5 min, 100 μ L plasma + 100 μ L roxithromycin in MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90, mix, add a 20-100 μ L aliquot to the SPE cartridge, wash SPE cartridge with MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90 at 0.5 mL/min, after 5 min backflush the contents of the SPE cartridge onto the column with the mobile phase, elute the column with the mobile phase and monitor the effluent.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Hypersil BDS C18

Mobile phase: MeCN:water 54:46 containing 4.5 mM NaH₂PO₄ and 6.8 mM Na₂HPO₄, pH 7

Column temperature: 55

Flow rate: 1

Detector: E, ESA Coulochem II, Model 5011 dual analytical cell, upstream +0.65 V, downstream +0.85 V (monitored), analytical cell protected by an ESA carbon in-line filter

CHROMATOGRAM

Retention time: 6

Internal standard: roxithromycin (7)

Limit of quantitation: 500 nM

KEY WORDS

plasma; SPE

REFERENCE

Hedenmo, M.; Eriksson, B.-M. Liquid chromatographic determination of the macrolide antibiotics roxithromycin and clarithromycin in plasma by automated solid-phase extraction and electrochemical detection. *J.Chromatogr.A*, **1995**, 692, 161–166

SAMPLE

Matrix: blood

Sample preparation: 500 μL Serum + 50 μL 15 μg/mL IS in MeCN:water 50:50 + 0.2 g (?) sodium carbonate + 3 mL hexane:ethyl acetate 50:50, vortex for 1 min, centrifuge at 800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL mobile phase, sonicate for 1 min, inject a 75 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Supelguard C8 (Supelco)

Column: 150 × 4.6 5 μm Supelcosil C8

Mobile phase: MeCN:MeOH:25 mM acetic acid 46:10:44 adjusted to pH 6.8 with NaOH

Flow rate: 1

Injection volume: 50

Detector: E, ESA Coulochem 5100A, 5010 analytical cell, +0.78 V

CHROMATOGRAM

Retention time: 17

Internal standard: erythromycin A-6-O-methyloxime (25)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; pharmacokinetics

REFERENCE

Nilsen, O.G.; Aamo, T.; Zahlsen, K.; Svarva, P. Macrolide pharmacokinetics and dose scheduling of roxithromycin. *Diagn.Microbiol.Infect.Dis.*, **1992**, 15, 71S–76S

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μL Plasma + 75 μL 10 $\mu\text{g}/\text{mL}$ IS in 1:1 MeCN:water + 200 μL 100 mM sodium carbonate + 3 mL ethyl acetate:hexane 1:1, stir vigorously for 1 min, centrifuge at 800 g for 5 min. Evaporate organic layer to dryness at 45° under a stream of air, dissolve residue in 200-400 μL 1:1 MeCN:water, inject 20-80 μL aliquot. Urine. 200 μL Urine + 300 μL 10 $\mu\text{g}/\text{mL}$ IS in 1:1 MeCN:water + 100 μL 100 mM sodium carbonate + 3-4 mL ethyl acetate:hexane 1:1, stir vigorously for 1 min on a vortex mixer, centrifuge at 800 g for 5 min. Evaporate organic layer to dryness at 45° under a stream of air, dissolve residue in 800-1200 μL 1:1 MeCN:water, inject aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Nucleosil C8

Mobile phase: MeCN:MeOH:water 39:9:52, containing 0.04 M NaH_2PO_4 and NaOH to bring the pH to 6.8

Flow rate: 1.2-1.4

Injection volume: 20-80

Detector: E, Environmental Sciences Assoc. Model 5100A, screening electrode +0.5 V, working electrode +0.78 \pm 0.04 V

CHROMATOGRAM

Retention time: 21

Internal standard: erythromycin A 9-O-methyloxime

Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, 14-hydroxyclearithromycin

KEY WORDS

plasma

REFERENCE

Chu, S.-Y.; Sennello, L.T.; Sonders, R.C. Simultaneous determination of clarithromycin and (14R)-hydroxyclearithromycin in plasma and urine using high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1991**, 571, 199-208

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.6 ODS-80TM (Tosoh)

Mobile phase: MeCN:67 mM KH_2PO_4 35:65

Column temperature: 50

Flow rate: 1

Detector: UV 210

REFERENCE

Ishii, K.; Katayama, Y.; Itai, S.; Ito, Y.; Hayashi, H. *In vitro* dissolution tests corresponding to the *in vivo* dissolution of clarithromycin tablets in the stomach and intestine. *Chem.Pharm.Bull.*, **1995**, 43, 1943-1948

ANNOTATED BIBLIOGRAPHY

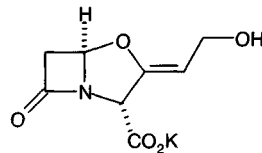
Morgan, D.K.; Brown, D.M.; Rotsch, T.D.; Plaszc, A.C. A reversed-phase high-performance liquid chromatographic method for the determination and identification of clarithromycin as the drug substance and in various dosage forms. *J.Pharm.Biomed.Anal.*, **1991**, 9, 261-269

Clavulanic Acid

Molecular formula: C₈H₉NO₅

Molecular weight: 199.2

CAS Registry No.: 58001-44-8 (clavulanic acid), 61177-45-5
(clavulanate potassium)



SAMPLE

Matrix: blood

Sample preparation: Filter (Amicon MPS-1 with YMT membrane) while centrifuging at 1500 g for 10 min, inject a 20 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μ m Develosil ODS-10

Column: 7 μ m Zorbax ODS-7

Mobile phase: MeOH:buffer 1:2.7 (1:3.5 for concentrations <100 ng/mL) (Prepare buffer by dissolving 1.791 g Na₂HPO₄·12H₂O and 0.780 g NaH₂PO₄·2H₂O in 1 L water, add tetrabutylammonium bromide to a final concentration of 5 mM.)

Flow rate: 0.8

Injection volume: 20

Detector: UV 272 following post-column reaction. The column effluent mixed with MeOH: 500 mM NaOH 1:2.7 (1:3.5 for concentrations <100 ng/mL) pumped at 0.2 mL/min and this mixture flowed through a 1 m \times 0.5 mm ID coil to the detector.

CHROMATOGRAM

Retention time: 5

Limit of detection: 25 ng/mL

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Noninterfering: ampicillin, cefoperazone, ticarcillin

KEY WORDS

plasma; post-column reaction; ultrafiltrate

REFERENCE

Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. Improved high-performance liquid chromatographic assay of clavulanic acid and sulbactam by postcolumn alkaline degradation. *J.Liq.Chromatogr.*, 1985, 8, 2521-2534

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize (Ultra-Turrax) 300 mg tissue at 4° for 45 s, centrifuge. 400 μ L Serum or tissue homogenate supernatant + 100 mM pH 6.8 ammonium citrate containing 3 M imidazole, vortex for 30 s, add 1 mL MeCN, mix for 15 s, centrifuge at 3000 rpm for 10 min. Add 3 mL dichloromethane to the supernatant, vortex for 30 s, centrifuge at 3000 rpm for 5 min, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 25 \times 4 5 μ m LiChrospher RP 18 E

Column: 125 \times 4 5 μ m LiChrospher RP 18 E

Mobile phase: MeCN:10 mM pH 3.2 KH₂PO₄ 4:96

Flow rate: 1.3

Injection volume: 50

Detector: UV 311

CHROMATOGRAM

Limit of detection: 100 ng/mL

KEY WORDS

serum; fat; colon

REFERENCE

Martin, C.; Mallet, M.-N.; Sastre, B.; Viviand, X.; Martin, A.; De Micco, P.; Gouin, F. Comparison of concentrations of two doses of clavulanic acid (200 and 400 milligrams) administered with amoxicillin (2,000 milligrams) in tissues of patients undergoing colorectal surgery. *Antimicrob. Agents Chemother.*, **1995**, *39*, 94–98

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 1 mL Serum + 1 mL MeCN, shake at 0° or 15 min, centrifuge at 8000 g for 10 min. Add supernatant to 10 mL dichloromethane, shake at 0° or 15 min, centrifuge at 8000 g for 10 min, discard organic layer. 200 μ L Aqueous layer + 400 μ L reagent, after 5 min inject 40 μ L aliquot. Urine. Dilute 10-fold, take 200 μ L + 400 μ L reagent, after 5 min inject 40 μ L aliquot. (Reagent was 3.45 g 1,2,4-triazole dissolved in 15 mL water, adjust pH to 7.0 with 4 M NaOH, make up to 25 mL.)

HPLC VARIABLES

Guard column: 50 \times 4.6 5 μ m Spherisorb C-18

Column: 250 \times 4.6 5 μ m Spherisorb C-18

Mobile phase: Gradient. MeCN:20 mM pH 7.0 phosphate buffer from 2:98 to 25:75 over 25 min

Flow rate: 0.5

Injection volume: 40

Detector: UV 315

CHROMATOGRAM

Retention time: 19

Limit of detection: 50 ng/mL

KEY WORDS

serum; derivatization

REFERENCE

Shah, A.J.; Adlard, M.W.; Stride, J.D. A sensitive assay for clavulanic acid and sulbactam in biological fluids by high-performance liquid chromatography and precolumn derivatization. *J. Pharm. Biomed. Anal.*, **1990**, *8*, 437–443

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 500 μ L Serum + 200 μ L 200 mM pH 7.0 phosphate buffer, vortex for 10 s, filter (Amicon YMT membrane) while centrifuging at 5° at 1500 g for 15 min. Mix 250 μ L ultrafiltrate with 250 μ L reagent, heat at 30° for 5 min, inject a 50 μ L aliquot. 500 μ L Urine + 4.5 mL water, mix vigorously for 20 s, filter (0.45 μ m) an aliquot. Mix 250 μ L filtrate with 250 μ L reagent, heat at 30° for 5 min, inject a 50 μ L aliquot. (Prepare reagent by dissolving 13.81 g 1,2,4-triazole in 70 mL water and adjusting the pH to 9.00 \pm 0.05 with 4 M NaOH, make up to 100 mL.)

HPLC VARIABLES

Guard column: 30 × 4.6 μBondapak C18

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:30 mM pH 7.0 phosphate buffer 20:80

Flow rate: 2

Injection volume: 50

Detector: UV 313

CHROMATOGRAM

Retention time: 7

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: degradation products, penicillins, penicilloic acids

KEY WORDS

serum; derivatization

REFERENCE

Martín, J.; Méndez, R. High-performance liquid chromatographic determination of clavulanic acid in human serum and urine using a pre-column reaction with 1,2,4-triazole. *J.Liq.Chromatogr.*, **1988**, *11*, 1697-1705

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 150-200 μL Plasma ultrafiltered (Amicon MPS-1, YMT membrane) at 1500 g for 10 min. 50 μL Ultrafiltrate + 150 μL 1 M pH 3.8 phosphate buffer + 20 μL 2% benzaldehyde in MeOH, heat at 100° for 20 min, cool to room temperature, inject 20-50 μL aliquot. Urine. Dilute 10-fold with water, filter (0.45 μm). 100 μL Filtrate + 300 μL 1 M pH 3.8 phosphate buffer + 40 μL 2% benzaldehyde in MeOH, heat at 100° for 20 min, cool to room temperature, inject 20-50 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Develosil ODS-5

Column: 150 × 4.6 5 μm Develosil ODS-5

Mobile phase: MeOH:water 1:1

Flow rate: 0.8

Injection volume: 20-50

Detector: F ex 386 em 460

CHROMATOGRAM

Retention time: 9

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin

Noninterfering: ticarcillin

KEY WORDS

plasma

REFERENCE

Haginaka, J.; Yasuda, H.; Uno, T.; Nakagawa, T. High-performance liquid chromatographic assay of clavulanate in human plasma and urine by fluorimetric detection. *J.Chromatogr.*, **1986**, *377*, 269-277

SAMPLE**Matrix:** blood, urine**Sample preparation:** Serum. 500 μL Serum + 500 μL 100 mM pH 7.0 phosphate buffer, mix, filter (Amicon MPS-1 ultrafiltration) with centrifugation at 4° and 1500 g for 20 min. Remove 100 μL ultrafiltrate, add 100 μL reagent, mix, inject a 75 μL aliquot taken from the top 5 mm. Urine. 100 μL urine + 100 μL reagent, mix, inject a 75 μL aliquot taken from the top 5 mm. (Reagent prepared by dissolving 8.25 g imidazole in 24 mL water + 2 mL 5 M HCl, adjust pH to 6.8 with 5 M HCl, make up to 40 mL with water.)

HPLC VARIABLES**Column:** 250 \times 4.6 5 μm Spherisorb ODS**Mobile phase:** MeOH:100 mM KH_2PO_4 + 50 mM pentanesulfonic acid + 100 mM ethanolamine 10:90**Flow rate:** 1.5**Injection volume:** 75**Detector:** UV 313

CHROMATOGRAM**Retention time:** 7**Limit of detection:** 100 ng/mL

KEY WORDS

serum; derivatization

REFERENCEWatson, I.D. Clavulanate-potentiated ticarcillin: high-performance liquid chromatographic assays for clavulanic acid and ticarcillin isomers in serum and urine. *J.Chromatogr.*, **1985**, 337, 301–309

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. Vortex plasma with 2 volumes of MeCN for 30 s, centrifuge at 3600 rpm for 5 min, inject a 50 μL aliquot of the supernatant. Urine. Filter (0.45 μm) urine, inject a 25 μL aliquot of the filtrate.

HPLC VARIABLES**Guard column:** 50 \times 4.6 LiChrosorb RP-2**Column:** 250 \times 4.6 Develosil ODS-10 (Nomura Chemicals)**Mobile phase:** MeOH:buffer A 25:75 (plasma) or MeOH:buffer B 20:100 (urine) (Buffer A was 0.1 mM Na_2HPO_4 containing 0.1 mM NaH_2PO_4 and 5 mM tetrabutylammonium bromide. Buffer B was 1 mM Na_2HPO_4 containing 1 mM NaH_2PO_4 and 5 mM tetrabutylammonium bromide.)**Flow rate:** 1.2**Injection volume:** 25-50**Detector:** UV 270 following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.6 mL/min and the mixture flowed through a 2 m \times 0.25 mm ID coil to the detector.)

CHROMATOGRAM**Retention time:** 13 (plasma), 20 (urine)**Limit of detection:** 100 ng/mL

KEY WORDS

post-column reaction; plasma

REFERENCE

Haginaka, J.; Yasuda, H.; Uno, T.; Nakagawa, T. Alkaline degradation of clavulanic acid and high performance liquid chromatographic determination by post-column alkaline degradation. *Chem. Pharm. Bull.*, **1983**, *31*, 4436-4447

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. Wash Amicon YMB filter membrane by stirring gently in 200 mL 100 mM pH 7.0 sodium phosphate buffer for 30 min, blot dry with filter paper. Dilute serum with an equal volume of 100 mM pH 7.0 sodium phosphate buffer, filter (Amicon YMB) while centrifuging at 5° at 1000 g for 15 min. Add 1 part reagent to 4 parts ultrafiltrate, let stand for 10 min, inject a 25-50 µL aliquot. Urine. Dilute 10-fold with 100 mM pH 7.0 sodium phosphate buffer. Add 1 part reagent to 4 parts diluted urine, let stand for 10 min, inject a 25-50 µL aliquot. (Reagent was 8.25 g imidazole, 24 mL water, and 2 mL 5 M HCl made up to 40 mL with water.)

HPLC VARIABLES

Guard column: CO:PEL ODS C18

Column: 250 × 4.6 µBondapak C18

Mobile phase: MeOH:buffer 6:94 (Use 4:96 for clavulanic acid concentrations of <2 µg/mL in urine.) (Buffer was 100 mM KH₂PO₄ adjusted to pH 3.2 with phosphoric acid.)

Flow rate: 2.5

Injection volume: 25-50

Detector: UV 311

CHROMATOGRAM

Retention time: 4

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: degradation products, amoxicillin

KEY WORDS

derivatization; ultrafiltrate; serum; pharmacokinetics

REFERENCE

Foulstone, M.; Reading, C. Assay of amoxicillin and clavulanic acid, the components of Augmentin, in biological fluids with high-performance liquid chromatography. *Antimicrob. Agents Chemother.*, **1982**, *22*, 753-762

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 NaH₂PO₄ 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

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water and filter (reject first few mL of filtrate)

HPLC VARIABLES

Column: 300 × 4 μ-Bondapak 10 μm C18

Mobile phase: MeOH:buffer:water 15:1:84 (Buffer was 50 mL 200 mM KH₂PO₄ + 5.7 mL 200 mM NaOH made up to 200 mL, pH 6.)

Flow rate: 1

Injection volume: 20

Detector: UV 235

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

Simultaneous: amoxicillin

KEY WORDS

tablets; suspensions

REFERENCE

Abounassif, M.A.; Abdel-Moety, E.M.; Mohamed, M.E.; Gad-Kariem, R.A. Liquid chromatographic determination of amoxycillin and clavulanic acid in pharmaceutical preparations. *J.Pharm. Biomed.Anal.*, 1991, 9, 731-735

SAMPLE

Matrix: formulations

Sample preparation: Dilute, filter, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 3.9 5μm Nova Pak C18

Mobile phase: MeOH:50 mM pH 4.0 phosphate buffer 3:97

Flow rate: 0.8

Detector: UV 254

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Simultaneous: amoxicillin

Noninterfering: degradation products, mannitol, saccharin

KEY WORDS

oral suspensions; stability-indicating

REFERENCE

Tu, Y.H.; Stiles, M.L.; Allen, L.V., Jr.; Olsen, K.M.; Barton, C.I.; Greenwood, R.B. Stability of amoxicillin trihydrate-potassium clavulanate in original containers and unit dose oral syringes. *Am.J. Hosp.Pharm.*, **1988**, *45*, 1092–1099

SAMPLE

Matrix: urine

Sample preparation: Dilute 10-fold with water, filter (0.45 μm acrylate copolymer), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μm Develosil ODS-10

Column: 150 \times 4.6 5 μm Develosil ODS-5 (Nomura Chemicals)

Mobile phase: MeOH:buffer 1:2.5 (Prepare buffer by dissolving 1.791 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 0.780 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in 5 L water, add tetrabutylammonium bromide to a final concentration of 5 mM.)

Flow rate: 0.8

Injection volume: 20

Detector: UV 272 following post-column reaction. The column effluent mixed with MeOH: 500 mM NaOH 1:2.5 pumped at 0.2 mL/min and this mixture flowed through a 1 m \times 0.5 mm ID coil to the detector.

CHROMATOGRAM

Retention time: 7

Limit of detection: 500 ng/mL

Limit of quantitation: 1 $\mu\text{g}/\text{mL}$

OTHER SUBSTANCES

Noninterfering: ampicillin, cefoperazone, ticarcillin

KEY WORDS

post-column reaction

REFERENCE

Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. Improved high-performance liquid chromatographic assay of clavulanic acid and sulbactam by postcolumn alkaline degradation. *J.Liq.Chromatogr.*, **1985**, *8*, 2521–2534

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Eckers, C.; Hutton, K.A.; de Biasi, V.; East, P.B.; Haskins, N.J.; Jacewicz, V.W. Determination of clavam-2-carboxylate in clavulanate potassium and tablet material by liquid chromatography-tandem mass spectrometry. *J.Chromatogr.*, **1994**, *686*, 213–218 [LC-MS; simultaneous impurities; bulk]

Jenke, D.R. Drug binding by reservoirs in elastomeric infusion devices. *Pharm.Res.*, **1994**, *11*, 984–989 [saline; 5% dextrose; also cilastatin, fluconazole, foscarnet, gentamicin, imipenem, lidocaine, penicillin G, tobramycin, vancomycin]

Low, A.S.; Taylor, R.B.; Gould, I.M. Determination of clavulanic acid by a sensitive HPLC method. *J.Antimicrob.Chemother.*, **1989**, *24 Suppl B*, 83–86

Haginaka, J.; Wakai, J.; Yasuda, H. Liquid chromatographic assay of β -lactamase inhibitors in human serum and urine using a hollow-fiber postcolumn reactor. *Anal.Chem.*, **1987**, *59*, 324–327 [serum; urine; post-column reaction]

Jehl, F.; Monteil, H.; Brogard, J.M. [Direct determination of clavulanic acid in biological fluids using HPLC]. *Pathol.Biol.(Paris)*, **1987**, *35*, 702–706

Bawdon, R.E.; Leveno, K.L.; Cunningham, F.G.; Nobles, B.; Nelson, S. Intrapartum pharmacokinetics of ticarcillin and clavulanic acid in serum as determined by high-pressure liquid chromatography. *Adv.Therapy*, **1984**, *1*, 419–426 [derivatization; SPE; serum; pharmacokinetics]

Haginaka, J.; Nakagawa, T.; Nishino, Y.; Uno, T. High performance liquid chromatographic determination of clavulanic acid in human urine. *J.Antibiot.(Tokyo)*, **1981**, *34*, 1189–1194

Clindamycin

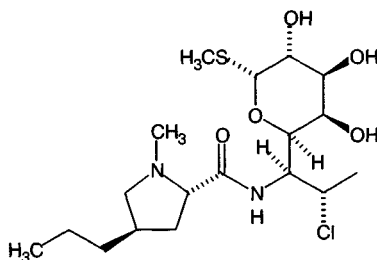
Molecular formula: C₁₈H₃₃ClN₂O₅S

Molecular weight: 425.0

CAS Registry No.: 18323-44-9, 58207-19-5 (HCl monohydrate),

36688-78-5 (palmitate), 25507-04-4

(palmitate HCl), 24729-96-2 (phosphate)



SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma or serum + 500 μ L 0.44 μ g/L triazolam in MeCN, vortex 20 s, centrifuge at 3000 g for 10 min, remove supernatant, evaporate under nitrogen to a volume of 250 μ L, inject 15-30 μ L aliquots

HPLC VARIABLES

Column: 150 \times 3.8 5 μ m Nova-Pak C18

Mobile phase: MeCN:water:85% phosphoric acid:7.6 mM tetramethylammonium chloride 30:70:0.2:0.075, final apparent pH 6.7 (adjusted with 1 M NaOH)

Flow rate: 1

Injection volume: 15-30

Detector: UV 198 (nitrogen purged)

CHROMATOGRAM

Retention time: 8.4

Internal standard: triazolam

OTHER SUBSTANCES

Simultaneous: clindamycin B, diazepam, mezlocillin, oxazepam, phenobarbital

Noninterfering: cefoperazone, cefotaxime, cephalothin, ticarcillin

KEY WORDS

plasma; serum; human; rabbit; dog; pig

REFERENCE

La Follette, G.; Gambertoglio, J.; White, J.A.; Knuth, D.W.; Lin, E.T. Determination of clindamycin in plasma or serum by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1988**, *431*, 379-388

SAMPLE

Matrix: bulk, formulations

Sample preparation: Capsules. Extract capsule contents with 0.5% phenethyl alcohol in mobile phase for 30 min, filter, inject an aliquot. Syrup. Measure out an amount of syrup containing 50 mg clindamycin, mix with 5 mL 0.5% phenethyl alcohol in mobile phase, inject an aliquot. Bulk. Dissolve 15 mg drug in 1 mL 0.5% phenethyl alcohol in mobile phase, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeOH:water:glacial acetic acid 60:40:0.2 containing 5 mM D, L-10-sodium camphor sulfonate, adjusted to pH 6.0 (RI detection) or MeOH:water 60:40 containing 10 mM phosphate buffer and 5 mM sodium pentanesulfonate, pH 6 (UV detection)

Flow rate: 1

Injection volume: 25

Detector: RI; UV 214

CHROMATOGRAM

Retention time: 11.3

Internal standard: phenethyl alcohol (5.9)

OTHER SUBSTANCES

Simultaneous: clindamycin B, 7-epiclindamycin

Noninterfering: lincomycin

KEY WORDS

capsules; syrup

REFERENCE

Landis, J.B.; Grant, M.E.; Nelson, S.A. Determination of clindamycin in pharmaceuticals by high-performance liquid chromatography using ion-pair formation. *J.Chromatogr.*, **1980**, *202*, 99–106

SAMPLE

Matrix: formulations

Sample preparation: Dilute 1:10 with 5% dextrose, remove a 900 μL aliquot and add it to 100 μL 1 mg/mL IS in MeOH, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Nucleosil C18

Mobile phase: MeOH:10 mM pH 6.3 phosphate buffer 40:60

Flow rate: 1.5

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 7.5

Internal standard: 2-nitrobenzenesulfonamide

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

injections; 5% dextrose; stability-indicating

REFERENCE

Sarkar, M.A.; Rogers, E.; Reinhard, M.; Wells, B.; Karnes, H.T. Stability of clindamycin phosphate, ranitidine hydrochloride, and piperacillin sodium in polyolefin containers. *Am.J.Hosp.Pharm.*, **1991**, *48*, 2184–2186

SAMPLE

Matrix: formulations

Sample preparation: 100 μL solution + 2 mL 20 $\mu\text{g}/\text{mL}$ propyl paraben in water, mix 15 s, inject 10 μL aliquot

HPLC VARIABLES

Column: 300 \times 4.1 10 μm Versapak C18

Mobile phase: MeOH:10 mM phosphate buffer + 5 mM pentanesulfonic acid 50:50, final pH 6.3

Flow rate: 1.2

Injection volume: 10

Detector: UV 214

CHROMATOGRAM

Retention time: 9

Internal standard: propyl paraben

OTHER SUBSTANCES

Noninterfering: degradation products, aztreonam, ceftazidime, ceftriaxone, piperacillin

KEY WORDS

injections; 5% dextrose; saline

REFERENCE

Marble, D.A.; Bosso, J.A.; Townsend, R.J. Stability of clindamycin phosphate with aztreonam, ceftazidime sodium, ceftriaxone sodium, or piperacillin sodium in two intravenous solutions. *Am.J.Hosp.Pharm.*, **1986**, *43*, 1732–1736

SAMPLE

Matrix: formulations

Sample preparation: Dilute 100 μL with 2 mL 20 $\mu\text{g}/\text{mL}$ propyl paraben in water, vortex for 15 s, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.1 10 μm Versapak C18 (Alltech)

Mobile phase: MeOH:10 mM phosphate buffer containing 5 mM sodium pentanesulfonate 50:50, pH 6.3

Flow rate: 1.2

Injection volume: 10

Detector: UV 214

CHROMATOGRAM

Retention time: 9

Internal standard: propyl paraben (15)

KEY WORDS

injections; saline; 5% dextrose

REFERENCE

Bosso, J.A.; Townsend, R.J. Stability of clindamycin phosphate and ceftizoxime sodium, cefoxitin sodium, cefamandole nafate, or cefazolin sodium in two intravenous solutions. *Am.J.Hosp.Pharm.*, **1985**, *42*, 2211–2214

SAMPLE

Matrix: solutions

Sample preparation: Dilute solution 10-fold with mobile phase. Mix 100 μL + 100 μL 50 $\mu\text{g}/\text{mL}$ propyl paraben, inject 10 μL .

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak}$ C18

Mobile phase: MeOH:10 mM KH_2PO_4 + 5 mM 1-pentanesulfonic acid 55:45

Flow rate: 1.2

Injection volume: 10

Detector: UV 214

CHROMATOGRAM**Retention time:** 8.1**Internal standard:** propyl paraben

KEY WORDSwater; stability-indicating

REFERENCE

Mahata, M.C.; Morosco, R.S.; Hipple, T.F. Stability of cimetidine hydrochloride and of clindamycin phosphate in water for injection stored in glass vials at two temperatures. *Am.J.Hosp.Pharm.*, **1993**, *50*, 2559–2561

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 \times 3.9 5 μm NOVA PAK C18**Mobile phase:** MeCN:50 mM pH 6.0 KH_2PO_4 35:65**Flow rate:** 1**Detector:** UV 214

CHROMATOGRAM**Retention time:** 3.8

REFERENCE

Koga, H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes. *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 400 $\mu\text{g}/\text{mL}$ solution of clindamycin in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Zorbax C8**Mobile phase:** MeCN:water 12:88 containing 0.25 g/L tetrabutylammonium perchlorate and 2 mL/L 70% perchloric acid, apparent pH adjusted to 2.5 with 50% NaOH**Flow rate:** 1.5**Injection volume:** 25**Detector:** UV 214

CHROMATOGRAM**Retention time:** 22.5

OTHER SUBSTANCES**Simultaneous:** benzyl alcohol, lincomycin, pirlimycin

REFERENCE

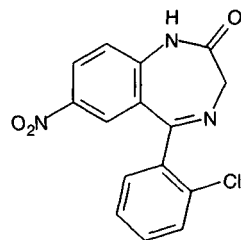
Theis, D.L. Ion-pairing liquid chromatographic method for the determination of pirlimycin hydrochloride. *J.Chromatogr.*, **1987**, *402*, 335–343

Clonazepam

Molecular formula: C₁₅H₁₀ClN₃O₃

Molecular weight: 315.7

CAS Registry No.: 1622-61-3



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Whole blood + 15 μ L 10 μ g/mL demoxepam, mix, add 500 μ L 200 mM pH 11.5 carbonate buffer, mix, add 6 mL butyl chloride, rotate gently for 20 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Nova-Pak phenyl

Mobile phase: MeCN:40 mM KH₂PO₄ 28:72, pH 3.75

Flow rate: 0.8

Injection volume: 50

Detector: UV 240

CHROMATOGRAM

Retention time: 16

Internal standard: demoxepam (8)

Limit of detection: 6 ng/mL;

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: 7-aminoclonazepam, flunitrazepam, nitrazepam

Simultaneous: alprazolam, bromazepam, carbamazepine, carbamazepine epoxide, desalkylflurazepam, desmethyldiazepam, diazepam, flurazepam, lorazepam, moclobemide, naproxen, oxazepam, phenobarbital, phenytoin, quinidine, quinine, salicylic acid, temazepam, triazolam

Noninterfering: acetaminophen, theophylline

KEY WORDS

whole blood

REFERENCE

Robertson, M.D.; Drummer, O.H. High-performance liquid chromatographic procedure for the measurement of nitrobenzodiazepines and their 7-amino metabolites in blood. *J.Chromatogr.B*, **1995**, *667*, 179-184

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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)) KH₂PO₄ 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:** 3.92**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benzepiril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, cibenzoline, cicletanine, clemastine, clomipramine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisroquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, dosulepine, doxepin, doxylamine, droperidol, etodolac, fenfluramine, fenpropfen, fentiazac, flecainide, floctafenine, flumazenil, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoprolol, mexiletine, mianserine, midazolam, moclobemide, moperone, morphine, nadolol, nalbuphine, naltorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pipamperone, piroxicam, prazepam, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfimpyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, toloxatone, trazodone, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, videsine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: chlorpropamide, clobazam, clonidine, disopyramide, ephedrine, estazolam, flunitrazepam, glipizide, melphalan, metoclopramide, minoxidil, pindolol, prazosin, tolbutamide, triazolam

KEY WORDSwhole blood; plasma

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:** Rock 5 mL whole blood + 10 mL water + 8.5 mL Na₂WO₄ in a 50 mL stoppered tube for 1 min, add 6 mL NiCl₂, rock for 5 min, add 15 mL 1-chlorobutane:isobutyl alcohol:THF 40:40:20, 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. (Na₂WO₄ prepared by mixing 10 g Na₂WO₄·2H₂O in 38 mL of 2 M NaOH and 2.5 g of NaHCO₃ and making up to 100 mL. NiCl₂ was 17% w/v NiCl₂ in water.)

HPLC VARIABLES**Column:** 200 × 4.6 5 μm Hypersil C8**Mobile phase:** Gradient. A was MeCN. B was 20 mM n-hexylamine adjusted to pH 4 with 85% phosphoric acid. A:B from 25:75 to 40:60 over 25 min to 50:50 over another 5 min**Injection volume:** 20**Detector:** UV 230

CHROMATOGRAM**Retention time:** 14**Limit of detection:** 0.30 ppm

OTHER SUBSTANCES**Extracted:** bromazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam**Also analyzed:** buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine

KEY WORDS

whole blood

REFERENCEBernal, 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 12 mL 500 mg PrepSep C1 SPE cartridge with 3 mL MeOH and 3 mL water. Add 1 mL plasma to SPE cartridge, wash with two 3 mL portions of water, wash with two 1 mL portions of MeOH:water 30:70, elute with two 1 mL portions of MeOH:50 mM pH 9.0 (NH₄)H₂PO₄ 90:10, evaporate the eluents under vacuum, dissolve the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES**Column:** 100 × 4.6 5 μm Spherisorb C8**Mobile phase:** MeCN:MeOH:20 mM (NH₄)H₂PO₄ 5:35:60 containing 2 mL/L 200 mM tetrabutylammonium bromide, final pH adjusted to 4.10**Column temperature:** 30**Flow rate:** 1.5**Injection volume:** 100**Detector:** UV 254

CHROMATOGRAM**Retention time:** 12.4**Internal standard:** clonazepam

OTHER SUBSTANCES**Extracted:** midazolam

KEY WORDS

plasma; clonazepam is IS; SPE

REFERENCE

Mastey, V.; Panneton, A.-C.; Donati, F.; Varin, F. Determination of midazolam and two of its metabolites in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *655*, 305–310

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 \times 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 \times 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

OTHER SUBSTANCES

Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clorazepate, clotiazepam, desmethyloclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, loflazepate, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam

Noninterfering: carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid

Interfering: lorazepam

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: 1 mL Plasma + 30 μL 0.5 $\mu\text{g}/\text{mL}$ flunitrazepam in MeOH + 50 μL 1 M ammonium hydroxide, vortex, add 8 mL diethyl ether, mix 2 min, remove organic phase and dry with 0.5 g anhydrous sodium sulfate. Add a few grains of sodium chloride to the ether to prevent bumping and evaporate to dryness in a water bath at 55°. Dissolve residue in 25 μL MeOH and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 30 × 4.6 3 μm Perkin-Elmer C18

Mobile phase: MeCN:water 25:75

Flow rate: 2.5

Injection volume: 20

Detector: UV 306

CHROMATOGRAM

Retention time: 2.4

Internal standard: flunitrazepam

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Simultaneous: clobazam, diazepam

Noninterfering: caffeine, carbamazepine, esobarbital, ethosuximide, flurazepam, medazepam, nitrazepam, oxazepam, phenobarbital, phenytoin, primidone, theobromine, theophylline

Interfering: desmethylclobazam, lorazepam

KEY WORDS

plasma

REFERENCE

Valenza, T.; Rosselli, P. Rapid and specific high-performance liquid chromatographic determination of clonazepam in plasma. *J.Chromatogr.*, **1987**, *386*, 363–366

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 1 mL saturated sodium tetraborate + 100 ng flunitrazepam + 7.5 mL n-hexane:ethyl acetate 9:1, vortex 5 min, centrifuge 1000 g. Repeat extraction. Combine extracts, evaporate to dryness, dissolve in 120 μL mobile phase, inject 100 μL.

HPLC VARIABLES

Guard column: 20 mm Supelco 40 μm Pelliguard LC-8

Column: 150 × 4.6 5 μm Supelco reversed phase C8

Mobile phase: MeCN:1.75 mM HCl:50 mM sodium acetate 36:10:54

Flow rate: 1.5

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 5

Internal standard: flunitrazepam

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: clobazam, nitrazepam

KEY WORDS

serum

REFERENCE

Zilli, M.A.; Nisi, G. Simple and sensitive method for the determination of clobazam, clonazepam and nitrazepam in human serum by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *378*, 492–497

SAMPLE**Matrix:** blood**Sample preparation:** Prepare a C18 Bond-Elut column by rinsing with 2 column volumes of MeOH and 2 column volumes of water using vacuum. Place 100 μL IS solution then 1 mL serum on column, wash column with 2 column volumes of water, wash with 50 μL MeOH, elute with two 200 μL portions of MeOH. Combine eluents, evaporate to dryness under a stream of air at 45°, reconstitute with 40 μL MeOH, inject whole sample. (IS was 50 ng/mL methylclonazepam in 1 M glycine buffer (pH adjusted to 10.5 with NaOH).)

HPLC VARIABLES**Column:** 150 \times 4.6 5 μm Ultrasphere ODS C18**Mobile phase:** MeCN:20 mM pH 3.8 phosphate buffer 30:70**Column temperature:** 50**Flow rate:** 2**Injection volume:** 40**Detector:** UV 254

CHROMATOGRAM**Retention time:** 6**Internal standard:** methylclonazepam**Limit of detection:** 2 ng/mL**Limit of quantitation:** 5 ng/mL

OTHER SUBSTANCES**Simultaneous:** amitriptyline, amobarbital, butalbital, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, demoxepam, desipramine, diazepam, ethinamate, flurazepam, glutethimide, heptobarbital, hexobarbital, lidocaine, medazepam, meperidine, mephobarbital, mesoridazine, methaqualone, pentathal, nortriptyline, oxazepam, pentobarbital, perphenazine, phenytoin, promazine, propranolol, protriptyline, quinidine, secobarbital**Noninterfering:** aprobarbital, barbituric acid, ethinamate, ethosuximide, gentamicin, lidocaine, mebutamate, meprobamate, methadone, methyprylon, nirvanol, phenobarbital, procainamide, propoxyphene, thioamyl, thioridazine, trifluoperazine, triflupromazine, tybamate, vinbarbital

KEY WORDS

serum

REFERENCEKabra, P.M.; Nzekwe, E.U. Liquid chromatographic analysis of clonazepam in human serum with solid-phase (Bond-Elut) extraction. *J.Chromatogr.*, **1985**, *341*, 383–390

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 \times 4 30 μm LiChocart Aluspher RP-select B (Merck)**Column:** 125 \times 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; UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, 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, fenethylamine, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, lorazepam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyl dopa, 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, tripeleminamine, 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, urine

Sample preparation: Prepare urine samples by adjusting pH to 5.0 with 2 M HCl then hydrolyzing with 20 μL 20 000 U/mL β -D-glucuronidase at 37° for 5 h. 500 μL Plasma or urine + 15 μL 2.5 mg/L desmethylflunitrazepam in MeOH + 50 μL 0.5 M NaOH + 5 mL diethyl ether, agitate, centrifuge, evaporate to dryness at 45° under vacuum, take up residue in 100 μL MeOH, inject 25 μL aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μm Nova Pak C18

Mobile phase: MeCN:MeOH:buffer 30:10:60 adjusted to pH 5.7 with 0.1 M HCl (Buffer was 94 mL 0.2 M NaH_2PO_4 + 6 mL 0.2 M Na_2HPO_4 .)

Flow rate: 1.3

Injection volume: 25

Detector: UV 242

CHROMATOGRAM

Retention time: 4.43

Internal standard: desmethylflunitrazepam (3.65)

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Simultaneous: alprazolam, bromazepam, chlordiazepoxide, clobazam, diazepam, estazolam, flunitrazepam, lorazepam, medazepam, triazolam

Noninterfering: acepromazine, aceprometazine, aprobarbital, barbital, butabarbital, clothiazepam, heptabarbital, hexobarbital, lorazepam, prazepam

Interfering: nitrazepam, oxazepam

KEY WORDS

plasma

REFERENCE

Boukhabza, A.; Lugnier, A.A.; Kintz, P.; Mangin, P.; Chaumont, A.J. Simple and sensitive method for monitoring clonazepam in human plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1990**, *529*, 210–216

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 30 μ L aliquot of a solution in MeOH.

HPLC VARIABLES**Column:** 250 \times 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:** 6

OTHER SUBSTANCES**Simultaneous:** diazepam, dothiepin, dothiepin sulfoxide, nordiazepam, nordothiepin, nor-dothiepin sulfoxide

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 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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:** 6.45 (A), 6.02 (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, clonidine, clozapine, cocaine, codeine, colchicine, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, mocllobemide, 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, sulfapyrazone, 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

some 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 $\mu\text{g}/\text{mL}$ solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 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' 1.35

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, 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

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 KH₂PO₄ and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)**Flow rate:** 0.5-1**Detector:** UV 210; UV 245

CHROMATOGRAM**Retention time:** 6.6**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, carbamazepine, chlordiazepoxide, chlorprothixene, 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

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, 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, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxy-

mesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, 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, methylidopa, methylpropamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxypenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, 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, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triproilidine, 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

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Sallustio, B.C.; Kassapidis, C.; Morris, R.G. High-performance liquid chromatography determination of clonazepam in plasma using solid-phase extraction. *Ther. Drug Monit.*, **1994**, *16*, 174–178 [SPE; plasma; methylclonazepam (IS); column temp 40 ; LOQ 5 ng/mL; LOD 2 ng/mL; non-interfering bromazepam, medazepam; simultaneous alprazolam, clobazam, chlordiazepoxide, diazepam, flunitrazepam, lorazepam, midazolam, nitrazepam, oxazepam, temazepam]

Bonato, P.S.; Lanchote, V.L. A rapid procedure for the purification of biological samples to be analysed by high performance liquid chromatography. *J. Liq. Chromatogr.*, **1993**, *16*, 2299–2308 [also alben-dazole, carbamazepine, clonazepam, desalkylflurazepam, mebendazole, methaqualone]

Furuno, K.; Gomita, Y.; Araki, Y.; Fukuda, T. Clonazepam serum levels in epileptic patients determined simply and rapidly by high-performance liquid chromatography using a solid-phase extraction column. *Acta Med. Okayama.*, **1991**, *45*, 123–127

Noggle, F.T., Jr.; Clark, C.R.; DeRuiter, J. Liquid chromatographic separation of some common benzodiazepines and their metabolites. *J. Liq. Chromatogr.*, **1990**, *13*, 4005–4021 [also alprazolam, bromazepam, chlordiazepoxide, clobazam, clorazepate, demoxepam, diazepam, estazolam, fludiazepam, flunitrazepam, flurazepam, halazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam, triazolam]

Doran, T.C. Liquid chromatographic assay for serum clonazepam. *Ther. Drug Monit.*, **1988**, *10*, 474–479

Dusci, L.J.; Hackett, L.P. Simultaneous determination of clobazam, N-desmethyl clobazam and clonazepam in plasma by high performance liquid chromatography. *Ther. Drug Monit.*, **1987**, *9*, 113–116

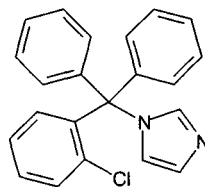
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Clotrimazole

Molecular formula: C₂₂H₁₇ClN₂

Molecular weight: 344.8

CAS Registry No.: 23593-75-1



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 μ L Plasma or whole blood + 50 μ L 100 μ M 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 μ L 95% EtOH, inject a 10 μ L aliquot of the eluate.

HPLC VARIABLES

Column: 50 \times 4.6 5 μ m Supelcosil LC-8DB

Mobile phase: MeOH:buffer 72.5:27.5 (Buffer was 25 mM K₂HPO₄ adjusted to pH 3 with 670 mM phosphoric acid.)

Flow rate: 1

Injection volume: 10

Detector: UV 210

CHROMATOGRAM

Retention time: 2.60

Internal standard: testosterone propionate (3.60)

OTHER SUBSTANCES

Extracted: metabolites, doxepin

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, mephentoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, par-amethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, procainamide, protriptyline, quinidine, theophylline, tobramycin, trimethadione, valproic acid, vancomycin

Interfering: itraconazole

KEY WORDS

plasma; SPE; whole blood; pharmacokinetics

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: Prepare a C18 SPE cartridge (Analytichem part 607303) by washing with 2 mL MeOH then 5 mL water (*J. Chromatogr.* 1986, 377, 287). 1 mL Serum + 200 μ L ammonium hydroxide, add to cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the residue to dryness under a stream of nitrogen at 45°, reconstitute in 1 mL mobile phase, inject 50 μ L aliquot.

HPLC VARIABLES**Column:** 150 × 3.9 Novopak C18**Mobile phase:** MeOH:MeCN:20 mM KH₂PO₄ 30:30:35, adjusted to pH 6.8**Flow rate:** 2**Injection volume:** 50**Detector:** UV 254

CHROMATOGRAM**Retention time:** 7.0**Internal standard:** clotrimazole

OTHER SUBSTANCES**Extracted:** ketoconazole

KEY WORDSserum; SPE; cotrimazole is IS

REFERENCE

Piscitelli, S.C.; Goss, T.F.; Wilton, J.H.; D'Andrea, D.T.; Goldstein, H.; Schentag, J.J. Effects of ranitidine and sucralfate on ketoconazole bioavailability. *Antimicrob. Agents Chemother.*, **1991**, *35*, 1765–1771

SAMPLE**Matrix:** blood, tissue

Sample preparation: Prepare a SPICE reversed-phase SPE cartridge by washing with 2 mL MeOH then 5 mL water. Tissue. 0.5 g Tissue + 4 mL MeCN, homogenize for 2 min using a PTFE pestle in a tissue grinder, centrifuge at 1500 g for 15 min. Remove supernatant and evaporate it under a stream of nitrogen at 45°. Reconstitute residue in 1 mL 10 mM HCl, add 200 µL ammonium hydroxide to adjust pH to about 10.5, add to cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the residue to dryness under a stream of nitrogen at 45°, reconstitute in 200 µL mobile phase, inject 50 µL aliquot. Plasma. 1 mL Plasma + 200 µL ammonium hydroxide, add to cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the residue to dryness under a stream of nitrogen at 45°, reconstitute in 1 mL mobile phase, inject 50 µL aliquot.

HPLC VARIABLES**Column:** 150 × 3.9 5 µm Novapak C18**Mobile phase:** MeCN:MeOH:20 mM pH 6.8 KH₂PO₄/NaOH 30:35:35**Flow rate:** 2**Injection volume:** 50**Detector:** UV 254

CHROMATOGRAM**Retention time:** 6.8**Internal standard:** clotrimazole

OTHER SUBSTANCES**Extracted:** ketoconazole

KEY WORDSplasma; SPE; clotrimazole is IS; lung; liver; adrenal

REFERENCE

Riley, C.M.; James, M.O. Determination of ketoconazole in the plasma, liver, lung and adrenal of the rat by high-performance liquid chromatography. *J. Chromatogr.*, **1986**, *377*, 287–294

SAMPLE**Matrix:** formulations**Sample preparation:** Dissolve in MeCN:THF 94.3:5.7, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Suplex pKb-100 (Supelco)**Mobile phase:** MeCN:THF:15 mM pH 4.1 acetate buffer 41.5:2.5:56**Flow rate:** 2**Detector:** UV 254

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES**Simultaneous:** degradation products, impurities, benzaldehyde, benzyl alcohol, mometa-
sone furoate, orthochlorophenyl-diphenylmethanol

KEY WORDS

creams

REFERENCESpangler, M. Isocratic reversed phase HPLC analysis of a pharmaceutical cream. *Supelco Reporter*,
1994, 13(2), 12-13

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 2 mL dichloromethane, elute with 3 mL MeOH:buffer 85:15. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH, inject 20 μL aliquot. (Buffer was 50 mM triethylamine adjusted to pH 7.0 with phosphoric acid.)

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 [Enhanced sensitivity with photoreactor (Beam Boost model C6808 with 10 m × 0.3 mm reaction coil) followed by UV detection at 270 nm.]

CHROMATOGRAM**Retention time:** 9.5**Internal standard:** ketoconazole (7)

OTHER SUBSTANCES**Simultaneous:** bifonazole, econazole, fenticonazole, isoconazole, miconazole, tioconazole

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: formulations

Sample preparation: 5 g Ointment containing 1% clotrimazole + 70 mL MeOH, sonicate for 15 min, make up to 100 mL with MeOH, filter (0.22 μ m PTFE), mix an aliquot of the filtrate with an equal volume of 6 μ g/mL chrysene in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 μ m Nucleosil C18

Mobile phase: MeOH:water 9:1

Flow rate: 1

Injection volume: 20

Detector: UV 258

CHROMATOGRAM

Retention time: 5.23

Internal standard: chrysene (9.54)

OTHER SUBSTANCES

Interfering: octanol

KEY WORDS

ointment

REFERENCE

Valenta, C.; Lexer, A.; Spiegl, P. Analysis of clotrimazole in ointments by high-performance liquid chromatography. *Pharmazie*, **1992**, *47*, 641–642

SAMPLE

Matrix: formulations

Sample preparation: 0.5 g Ointment containing 1% clotrimazole + 7 mL octanol, sonicate for 15 min, make up to 10 mL with octanol, filter (0.22 μ m PTFE), inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 μ m Nucleosil C18

Mobile phase: MeOH:4.25% phosphoric acid 9:1

Flow rate: 1

Injection volume: 20

Detector: UV 258

CHROMATOGRAM

Retention time: 2.33

KEY WORDS

ointment

REFERENCE

Valenta, C.; Lexer, A.; Spiegl, P. Analysis of clotrimazole in ointments by high-performance liquid chromatography. *Pharmazie*, **1992**, *47*, 641–642

ANNOTATED BIBLIOGRAPHY

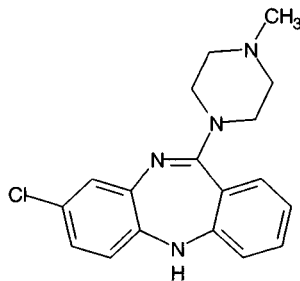
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Clozapine

Molecular formula: C₁₈H₁₉ClN₄

Molecular weight: 326.8

CAS Registry No.: 5786-21-0



SAMPLE

Matrix: bile, blood, urine

Sample preparation: 1 mL Bile, plasma, or urine + 20 μ L 40 μ g/mL clozapine in EtOH + 200 μ L 2.5 mM pH 6.0 sodium pentanesulfonate, mix for 3 s, add 6 mL diethyl ether, shake vigorously for 2 min, centrifuge at 2500 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 μ L mobile phase, vortex for 30 s, inject a 25 μ L aliquot.

HPLC VARIABLES

Guard column: 25 \times 4.6 30 μ m C18 (Merck)

Column: 100 \times 8 10 μ m μ Bondapak C18

Mobile phase: MeOH:water:triethylamine 65:35:0.5, adjust pH to 6-7 with glacial acetic acid

Flow rate: 2

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Retention time: 9.7

Internal standard: clozapine

OTHER SUBSTANCES

Extracted: moricizine

KEY WORDS

plasma; rat; clozapine is IS

REFERENCE

Yang, J.M.; Chan, K. Simultaneous determination of moricizine and its sulphoxidation metabolites in biological fluids by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 663, 172-176

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL buffer. Mix 100 (rat) or 250 (human) μ L plasma or serum with 100 μ L 5 μ g/mL N-methylspiperone, add to SPE cartridge, wash twice with 1 mL MeCN:buffer 28:72, elute with 1 mL MeOH:triethylamine 99.3:0.7. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 55 μ L aliquot. (Buffer was 7.8 g K₂HPO₄ in 1 L water, pH 9.0.)

HPLC VARIABLES

Guard column: 50 mm long C18 (Waters)

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:buffer 36:64, pH adjusted to 3.7 with 10% phosphoric acid (Buffer was 7.8 g K₂HPO₄ in 500 mL water, adjust pH to 4.0 with phosphoric acid, make up to 1 L with water.)

Flow rate: 1

Injection volume: 55

Detector: UV 254

CHROMATOGRAM

Retention time: 6.0

Internal standard: N-methylspiperone (11.2)

Limit of quantitation: 60 ng/mL (rat); 15 ng/mL (human)

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: amitriptyline, diazepam, nitrazepam, oxazepam, protriptyline

KEY WORDS

plasma; serum; human; rat; pharmacokinetics; SPE

REFERENCE

Fadiran, E.O.; Leslie, J.; Fossler, M.; Young, D. Determination of clozapine and its major metabolites in human serum and rat plasma by liquid chromatography using solid-phase extraction and ultra-violet detection. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 185–190

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 volume of 1 M HCl, 2 volumes of MeOH, and 1 volume of water. 500 μ L Serum + 50 μ L amoxapine in 1% potassium bicarbonate + 500 μ L MeCN, mix, centrifuge at 1500 g for 3 min, add the supernatant to the SPE cartridge, wash with 2 volumes of water, wash with 1 volume of MeCN, elute with 250 μ L MeOH:35% perchloric acid 100:1, remove all eluate by centrifuging at 1000 g for 30 s, inject a 15 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m C8 (Applied Biosystems)

Column: 150 \times 4.6 5 μ m Ultrasphere Octyl

Mobile phase: MeCN:0.1% tetramethylammonium perchlorate 27:73, adjusted to pH 4.2 with 10% perchloric acid

Flow rate: 2

Injection volume: 15

Detector: UV 245

CHROMATOGRAM

Retention time: 7.3

Internal standard: amoxapine (15.8)

Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; SPE; protect from light

REFERENCE

Gupta, R.N. Column liquid chromatographic determination of clozapine and N-desmethylozapine in human serum using solid-phase extraction. *J.Chromatogr.B*, **1995**, *673*, 311–315

SAMPLE**Matrix:** blood**Sample preparation:** 200 μ L Plasma or serum + 50 μ L 4 μ g/mL nortriptyline hydrochloride in 100 mM HCl + 100 μ L 2 M pH 10.6 Tris buffer + 200 μ L MTBE, vortex for 30 s, centrifuge at 9950 g for 3 min, inject a 50 μ L aliquot of the organic layer.

HPLC VARIABLES**Guard column:** 10 \times 4.6 SCX-10C5 (Hichrom)**Column:** 150 \times 4.6 Spherisorb S5 SCX (sulfopropyl-bonded silica) cation exchange**Mobile phase:** 35 mM ammonium perchlorate in MeOH adjusted to an apparent pH of 6.7 with 100 mM NaOH in MeOH**Flow rate:** 1.5**Injection volume:** 50**Detector:** UV 215

CHROMATOGRAM**Retention time:** 6**Internal standard:** nortriptyline hydrochloride (9)**Limit of detection:** 50 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites**Simultaneous:** amitriptyline, chlorpromazine, clomipramine, dothiepin, doxepin, fluoxetine, fluphenazine, haloperidol, imipramine, maprotiline, mianserin, norclomipramine, nordothiepin, nordoxepin, norfluoxetine, nortriptyline, paroxetine, remoxipride, sertraline, sulpride, thioridazine, trazodone**Noninterfering:** carbamazepine, clonazepam, diazepam, flunitrazepam, lorazepam, nordiazepam, theophylline**Interfering:** fluvoxamine

KEY WORDS

plasma; serum

REFERENCEMcCarthy, P.T.; Hughes, S.; Paton, C. Measurement of clozapine and norclozapine in plasma/serum by high-performance liquid chromatography with ultraviolet detection. *Biomed.Chromatogr.*, **1995**, *9*, 36-41

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m 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:** 6.53**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlor-diazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, cocaine, codeine, colchicine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nocardipine, nifedipine, niflumic acid, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfipyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, ti-neptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: bumadizone, cyamemazine, cyclizine, demexiptiline, diphenhydramine, histapyrridine, medazepam, nimodipine, phenylbutazone, proguanil, trifluoperidol

KEY WORDS

whole blood; plasma

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: Inject plasma onto column A and elute to waste with mobile phase A, elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 20 × 4.6 10 μm CN; B 250 × 4.6 5 μm Hypersil C18 ODS

Mobile phase: A water; B MeCN:water 40:60 containing 0.4% TEMED adjusted to pH 6.5 with glacial acetic acid

Detector: UV 254

CHROMATOGRAM

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

column-switching; plasma

REFERENCE

Weigmann, H.; Hiemke, C. Automated determination of clozapine, N-desmethylozapine and clozapine-N-oxide by column switching and on-line high-performance liquid chromatography (Abstract 110). *Ther.Drug Monit.*, **1995**, *17*, 410

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 4 μ g/mL protriptyline + 200 μ L 2 M NaOH, vortex, allow to stand for 5 min, add 5 mL n-hexane:isoamyl alcohol 98.5:1.5, shake for 20 min, centrifuge at 3000 g for 5 min. Remove the organic phase and add it to 1 mL 100 mM HCl, shake for 5 min, centrifuge at 3000 g for 2 min. Remove the aqueous layer and add it to 200 μ L 2 M NaOH, add n-hexane:isoamyl alcohol 98.5:1.5, extract, repeat extraction. Combine the organic layers and evaporate them at 45° under a stream of nitrogen, reconstitute in 100 μ L 100 mM HCl, inject a 80 μ L aliquot.

HPLC VARIABLES

Guard column: 70 mm Whatman column survival kit 10 μ m ODS

Column: 250 \times 4.6 10 μ m Partisil 10 ODS-3

Mobile phase: MeCN:MeOH:buffer 24:12:64 (Buffer was 5 g Na₂HPO₄ (per L?) adjusted to pH 4.0 with phosphoric acid.)

Flow rate: 2

Injection volume: 80

Detector: UV 230

CHROMATOGRAM

Retention time: 9

Internal standard: protriptyline (15)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Chung, M.-C.; Lin, S.-K.; Chang, W.-H.; Jann, M.W. Determination of clozapine and desmethylozapine in human plasma by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1993**, *613*, 168-173

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 1 mL 100 mM NaOH + 50 μ L 4.098 μ g/mL trifluoperazine and 2.801 μ g/mL imipramine in EtOH:water 1:1, mix, add 5 mL heptane:isooctyl alcohol 99:1, shake at 250 shakings/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/ethanol. Remove the organic layer and evaporate it to dryness at 50° under a stream of nitrogen, dissolve the residue in 75 μ L mobile phase, inject a 65 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Spherisorb S5W

Mobile phase: MeOH:50 mM pH 9.9 ammonium acetate buffer 85:15

Flow rate: 1.4

Injection volume: 65

Detector: UV 261

CHROMATOGRAM

Retention time: 2.3

Internal standard: trifluoperazine (3.5), imipramine (7.7)

Limit of detection: 15 ng/mL

Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, amitriptyline, carbamazepine, chlorprothixene, clomipramine, levomepromazine, nortriptyline

Interfering: mianserine, perphenazine, zuclopenthixol

KEY WORDS

serum

REFERENCE

Olesen, O.V.; Poulsen, B. On-line fully automated determination of clozapine and desmethylclozapine in human serum by solid-phase extraction on exchangeable cartridges and liquid chromatography using a methanol buffer mobile phase on unmodified silica. *J.Chromatogr.*, **1993**, 622, 39–46

SAMPLE

Matrix: blood

Sample preparation: Condition a 10 \times 2 SPE cartridge packed with 40 μ m cyanopropyl bonded phase (J.T. Baker) with MeOH at 1.5 mL/min for 1 min, with water at 4.5 mL/min for 2 min, with 100 mM pH 8.0 ammonium acetate buffer at 1.5 mL/min for 2 min. 600 μ L Serum + 300 μ L trifluoperazine and imipramine in water, mix, add a 200 μ L aliquot to the SPE cartridge, wash with 100 mM pH 8.0 ammonium acetate buffer at 1.5 mL/min for 0.5 min, wash with MeOH:water 9:91 at 1.5 mL/min for 1 min, elute the contents of the SPE cartridge directly onto the analytical column with mobile phase for 1 min.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Spherisorb S5W

Mobile phase: MeOH:50 mM pH 9.9 ammonium acetate buffer 85:15

Flow rate: 1.4

Detector: UV 261

CHROMATOGRAM

Retention time: 2.3

Internal standard: trifluoperazine (3.5), imipramine (7.7)

Limit of detection: 15 ng/mL

Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, amitriptyline, carbamazepine, chlorprothixene, clomipramine, levomepromazine, nortriptyline

Interfering: mianserine, perphenazine, zuclopenthixol

KEY WORDS

serum; SPE

REFERENCE

Olesen, O.V.; Poulsen, B. On-line fully automated determination of clozapine and desmethylclozapine in human serum by solid-phase extraction on exchangeable cartridges and liquid chromatography using a methanol buffer mobile phase on unmodified silica. *J.Chromatogr.*, **1993**, *622*, 39–46

SAMPLE

Matrix: blood

Sample preparation: 600 μ L Serum + 100 μ L 300 μ g/mL triprolidine in 400 mM NaOH + 7 mL ethyl acetate, vortex for 1 min, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 150 μ L 100 mM HCl, vortex for 1 min, centrifuge, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb-C6

Mobile phase: MeCN:30 mM KH_2PO_4 45:55 containing 2 g sodium hexanesulfonate, pH adjusted to 2.7 with phosphoric acid

Column temperature: 30

Flow rate: 1.5

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 4.5

Internal standard: triprolidine (5.5)

Limit of detection: 3-4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Interfering: carbinoxamine, chlorpheniramine, cocaine, dipyridamole, oxazolam, trazodone

KEY WORDS

serum

REFERENCE

Volpicelli, S.A.; Centorrino, F.; Puopolo, P.R.; Kando, J.; Frankenburg, F.R.; Baldessarini, R.J.; Flood, J.G. Determination of clozapine, norclozapine, and clozapine-N-oxide in serum by liquid chromatography. *Clin.Chem.*, **1993**, *39*, 1656–1659

SAMPLE

Matrix: blood

Sample preparation: Add 10 μ L 20 μ g/mL oxaprotiline in MeOH to 990 μ L plasma or serum. Inject 100 μ L 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: 6.2

Internal standard: oxaprotiline (9.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, imipramine, maprotiline, metoclopramide, norfluoxetine, nortriptyline

Noninterfering: carbamazepine, chlordiazepoxide, clobazam, diazepam, flurazepam, fluspirilene, haloperidol, lorazepam, nitrazepam, nordiazepam, oxazepam, perazine, pimo-
zide, spiroperidol, trifluoperidol

Interfering: fluvoxamine

KEY WORDS

plasma; serum; column-switching

REFERENCE

Härtter, S.; Wetzels, 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, saliva, tissue, urine

Sample preparation: Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5 NaH₂PO₄ 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 K₂HPO₄ containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)

Flow rate: 2

Injection volume: 75

Detector: UV 235

CHROMATOGRAM

Retention time: 5.6

Internal standard: 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, cocaine, gepirone, methylphenidate, pentazocine, pseudo-cocaine

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, methaqualone, 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, benztrapine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobamate, 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: enzyme incubations

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 2.5 μ m Ultracarb ODS 30 (Phenomenex)

Column: 100 \times 2.5 μ m Ultracarb ODS 30 (Phenomenex)

Mobile phase: MeCN:water:acetic acid 75:25:1 containing 1 mM ammonium acetate

Flow rate: 0.2

Injection volume: 20

Detector: UV 254; MS, Sciex API III triple quadrupole, IonSpray interface, ionizing voltage 5 kV, nebulizing gas air at 40 psi, orifice voltage 60 V, target gas argon 26 eV, post-column splitter to decrease flow to 20 μ L/min

OTHER SUBSTANCES

Extracted: metabolites, degradation products

REFERENCE

Liu, Z.C.; Uetrecht, J.P. Clozapine is oxidized by activated human neutrophils to a reactive nitrogen ion that irreversibly binds to the cells. *J.Pharmacol.Exp.Ther.*, **1995**, *275*, 1476-1483

SAMPLE

Matrix: microsomal incubations

Sample preparation: Microsomal incubation + 2 mL ice-cold MeOH, let stand at 4° for 16 h, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 150 μ L MeOH:water 50:50, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 3.2 5 μ m Nucleosil C8

Mobile phase: Gradient. MeCN:6 mM pH 3.5 ammonium formate from 10:90 to 25:75 over 15 min, to 55:45 over 20 min.

Flow rate: 0.7

Injection volume: 10

Detector: UV 254; MS, Fisons Quattro II quadrupole, column effluent passed through a splitter and 40 μ L/min passed through a 150 cm \times 75 μ m fused-silica capillary to the electrospray probe, nebulizing gas nitrogen at 12 L/h, drying gas nitrogen at 280 L/h, interface 60°, capillary voltage 4 kV, con (orifice) voltage 30-80 V, photomultiplier 550 V, m/z 327

CHROMATOGRAM

Retention time: 27

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; mouse; rat; liver

REFERENCE

Maggs, J.L.; Williams, D.; Pirmohamed, M.; Park, B.K. The metabolic formation of reactive intermediates from clozapine, a drug associated with agranulocytosis in man. *J.Pharmacol.Exp.Ther.*, **1995**, 275, 1463-1475

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 2 mL ice-cold MeOH, let stand at 4° overnight, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in EtOH:water 50:50, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Nucleosil 5 C8

Mobile phase: Gradient. MeCN:100 mM pH 3.8 ammonium acetate buffer from 20:80 to 40:60 over 15 min, return to 20:80 over 5 min.

Flow rate: 1.5

Injection volume: 50

Detector: UV 254; Radioactivity

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

liver

REFERENCE

Pirmohamed, M.; Williams, D.; Madden, S.; Templeton, E.; Park, B.K. Metabolism and bioactivation of clozapine by human liver *in vitro*. *J.Pharmacol.Exp.Ther.*, **1995**, 272, 984-990

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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: 10.90 (A), 5.15 (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, 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, methyl dopa, 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, sulfipyrazone, 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

some 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

ANNOTATED BIBLIOGRAPHY

Hubmann, M.R.; Waschler, R.; Conca, A.; König, P. Simultaneous drug monitoring of citalopram, clozapine, fluoxetine, maprotiline, and trazodone by HPLC analysis (Abstract 41). *Ther.Drug Monit.*, **1995**, 17, 393 [simultaneous citalopram, clozapine, fluoxetine, maprotiline, trazodone; LOQ 50 ng/mL]

- Schulz, E.; Fleischhaker, C.; Remschmidt, H. Determination of clozapine and its major metabolites in serum samples of adolescent schizophrenic patients by high-performance liquid chromatography. Data from a prospective clinical trial. *Pharmacopsychiatry*, **1995**, *28*, 20–25
- Lin, S.-K.; Chang, W.-H.; Chung, M.-C.; Lam, Y.W.F.; Jann, M.W. Disposition of clozapine and desmethylclozapine in schizophrenic patients. *J.Clin.Pharmacol.*, **1994**, *34*, 318–324 [plasma; protriptyline (IS); LOD 2 ng/mL]
- Weigmann, H.; Hiemke, C. Determination of clozapine and its major metabolites in human serum using automated solid-phase extraction and subsequent isocratic high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1992**, *583*, 209–216
- Lovdahl, M.J.; Perry, P.J.; Miller, D.D. The assay of clozapine and N-desmethylclozapine in human plasma by high-performance liquid chromatography. *Ther.Drug Monit.*, **1991**, *13*, 69–72
- Wilhelm, D.; Kemper, A. High-performance liquid chromatographic procedure for the determination of clozapine, haloperidol, droperidol and several benzodiazepines in plasma. *J.Chromatogr.*, **1990**, *525*, 218–224
- Humpel, C.; Haring, C.; Saria, A. Rapid and sensitive determination of clozapine in human plasma using high-performance liquid chromatography and amperometric detection. *J.Chromatogr.*, **1989**, *491*, 235–239
- Haring, C.; Humpel, C.; Auer, B.; Saria, A.; Barnas, C.; Fleischhacker, W.; Hinterhuber, H. Clozapine plasma levels determined by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1988**, *428*, 160–166
- Yin, J.L. [HPLC determination of clozapine in plasma]. *Chung Hua Shen Ching Ching Shen Ko Tsa Chih*, **1987**, *20*, 78–80
- Wang, Z.R.; Lu, M.L.; Xu, P.P.; Zeng, Y.L. Determination of clozapine and its metabolites in serum and urine by reversed phase HPLC. *Biomed.Chromatogr.*, **1986**, *1*, 53–57

Codeine

Molecular formula: C₁₈H₂₁NO₃

Molecular weight: 299.4

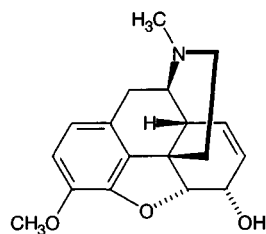
CAS Registry No.: 76-57-3, 6069-47-8 (monohydrate), 5913-71-3

(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)



SAMPLE

Matrix: bile, blood

Sample preparation: 0.5 mL Blood or bile + 10 (blood) or 15 (bile) μ L 100 μ g/mL nalorphine in MeOH + 300 μ L 1.1 M pH 5.0 sodium acetate buffer + 3000-3500 U of *Patella vulgata* glucuronidase, incubate at 55° overnight, add 0.5 mL borate buffer to achieve a pH of 8.3-8.5. Add 8 mL chloroform:isopropanol 90:10, gently rotate for 30 min, centrifuge at 3500 rpm for 10 min, remove aqueous layer. Wash organic layer (twice for blood, three times for bile) with 3 mL 100 mM pH 9.9 sodium phosphate buffer with gentle rotation for 10 min and centrifugation each time. Add organic layer to 200 (blood) or 400 (bile) μ L 0.2% phosphoric acid, gently rotate for 30 min, discard organic layer, inject 50 μ L of the acid layer. (Borate buffer was 50 mM boric acid and 43 mM sodium tetraborate, adjusted to pH 9.8.)

HPLC VARIABLES

Guard column: Nova-Pak phenyl guard column

Column: 150 \times 3.9 5 μ m Nova-Pak phenyl

Mobile phase: MeCN:10 mM pH 6.6 NaH₂PO₄ 10:90

Flow rate: 1.2

Injection volume: 50

Detector: UV 210; F ex 220 em 370 (cut-off)

CHROMATOGRAM

Retention time: 19.2

Internal standard: nalorphine (23.5)

Limit of detection: 60 ng/mL (blood), 200 ng/mL (bile)

OTHER SUBSTANCES

Simultaneous: dihydrocodeine, hydrocodone, 6-monoacetylmorphine, morphine, oxycodone

Noninterfering: acetylcodeine, amitriptyline, amphetamine, diamorphine, diazepam, dothiepin, doxepin, ephedrine, ephedrine, hydromorphone, mesoridazine, methadone, methamphetamine, 3-monoacetylmorphine, nordiazepam, norpropoxyphene, nortriptyline, oxazepam, propoxyphene, pseudoephedrine, quinidine, quinine, sulfamethoxazole, sulforidazine, thioridazine

KEY WORDS

UV and F detection used together

REFERENCE

Crump, K.L.; McIntyre, I.M.; Drummer, O.H. Simultaneous determination of morphine and codeine in blood and bile using dual ultraviolet and fluorescence high-performance liquid chromatography. *J.Anal.Toxicol.*, **1994**, *18*, 208-212

SAMPLE

Matrix: bile, blood, tissue

Sample preparation: 250 μ L Bile, 3 mL blood, or 5 mL tissue homogenate + 1 mL 200 μ g/mL nalorphine in water + 2 mL 200 mM pH 8.9 sodium borate buffer + 5 (bile) or 10 (blood, tissue) mL chloroform:isopropanol 90:10, rotate gently for 20 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer and add it to 2 mL 500 mM HCl, rotate for 20 min, centrifuge for 5 min. Remove 1.8 mL of the upper aqueous phase, adjust to pH 8.6 ± 0.2 by very carefully adding powdered ammonium carbonate until the solution is saturated, add 5 mL ethyl acetate:isopropanol 90:10, rotate for 20 min, centrifuge for 5 min. Remove 4.8 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μ L MeOH, vortex for 30 s, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 5 μ m RP-18 Spheri-5

Column: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeOH:50 mM pH 7 phosphate buffer 40:60 (Place a 70 \times 2 30-38 μ m Co-Pell ODS column before the injection valve.)

Column temperature: 50

Flow rate: 2

Injection volume: 20

Detector: E, Environmental Sciences Associates Model 5100, porous graphite electrode W1 900 mV W2 400 mV, difference in electrolysis current monitored

CHROMATOGRAM

Retention time: 8.95

Internal standard: nalorphine (14.72)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: hydromorphone, morphine, norcodeine, normorphine

Simultaneous: acetaminophen, atropine, epinephrine, ethylmorphine, hydrocodone, hydroxyzine, naloxone, oxycodone, pentazocine, phenylpropanolamine, pseudomorphine, scopolamine, secobarbital

Noninterfering: brompheniramine, chloroprocaine, dextromethorphan, diazepam, diphenhydramine, fentanyl, flurazepam, meperidine, methadone, neostigmine, propoxyphene

REFERENCE

Hepler, B.R.; Sutherland, C.; Sunshine, I.; Sebrosky, G.F. Combined enzyme immunoassay-LCEC method for the identification, confirmation, and quantitation of opiates in biological fluids. *J. Anal. Toxicol.*, 1984, 8, 78-90

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg ethyl SPE cartridge (J.T.Baker) with 2 volumes of MeOH, 1 volume of water, and 2 volumes of 10 mM pH 9.3 ammonium hydrogen carbonate buffer. 1 mL Serum + 100 μ L water, add to the SPE cartridge, wash with 1 volume of 10 mM ammonium hydrogen carbonate buffer, elute with 1 volume of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil ABZ

Mobile phase: Gradient. MeOH:water from 15:85 to 60:40 over 10 min. (Convex gradient where $\text{MeOH}\% = -0.46\exp(-x/1.18) + 0.6$ where $x = \text{time in min.}$)

Flow rate: 0.8 (0.018 mL/min entered MS)

Injection volume: 20

Detector: MS, Fisons TRIO 2, electrospray, capillary tip 2.97 kV, counter electrode 390 V, sampling cone voltages 66 V, -106 V, -17 V, source 60°, SIM m/z 300

CHROMATOGRAM**Retention time:** 7.03**Internal standard:** codeine**Limit of quantitation:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** morphine (m/z 286)

KEY WORDSserum; human; mouse; SPE, codeine is IS

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:** 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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m 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 286

CHROMATOGRAM**Retention time:** 3.44**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amitriptyline, amodiaquine, amoxapine, astemizole, atenolol, benazepril, benperidol, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cimetidine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin,

iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, loperamide, loperazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephentermine, mepivacaine, metapramine, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, naloxone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nifedipine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, reserpine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, suriclone, temazepam, tenoxicam, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiaprofenic acid, ticlopidine, timolol, tiocloमारol, tofisopam, tolbutamide, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, videsine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: albuterol, amisulpride, aspirin, benzocaine, chlormezanone, codeine, lisinopril, mephesisin, metformin, nalorphine, naltrexone, nizatidine, phenobarbital, phenol, ranitidine, ritodrine, sultopride, terbutaline, tiapride, toloxatone

KEY WORDS

whole blood; plasma

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 water. 1 mL Plasma + 100 μ L 1000 ng/mL nalorphine + 1 mL water, add to the SPE cartridge at 2 mL/min, wash with 2 mL water, wash with 2 mL MeCN, dry under vacuum for 1 min, elute with 2 mL dichloromethane:isopropanol:ammonium hydroxide 80:20:2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μ L mobile phase, inject a 60 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 3 μ m Basic C8 (YMC)

Mobile phase: MeCN:5 mM (NH₄)₂HPO₄ 8:92 adjusted to pH 5.8 with phosphoric acid

Flow rate: 1

Injection volume: 60

Detector: F ex 214 em 345

CHROMATOGRAM

Retention time: 15

Internal standard: nalorphine (18)

Limit of detection: 5 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: morphine, norcodeine

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Weingarten, B.; Wang, H.-Y.; Roberts, D.M. Determination of codeine in human plasma by high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.A*, **1995**, *696*, 83–92

SAMPLE

Matrix: blood

Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na₂WO₄ in a 50 mL stoppered tube for 1 min, add 6 mL NiCl₂, 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. (Na₂WO₄ prepared by mixing 10 g Na₂WO₄·2H₂O in 38 mL of 2 M NaOH and 2.5 g of NaHCO₃ and making up to 100 mL. NiCl₂ was 17% w/v NiCl₂ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8

Mobile phase: Gradient. A was MeCN. B was 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: 11

Limit of detection: 0.20 ppm

OTHER SUBSTANCES

Extracted: buprenorphine, caffeine, cocaine, 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: 1 mL plasma + IS, vortex 30 s, add 1 mL 50 mM pH 8 phosphate buffer, vortex, add 6 mL hexane:dichloromethane 2:1, shake 5 min, centrifuge, repeat extraction. Combine organic phases and extract with 1 mL 50 mM pH 3 acetate buffer. Make aqueous phase alkaline with 1 mL 0.1 M NaOH and extract with 6 mL hexane:dichloromethane 2:1. Evaporate organic phase to dryness, dissolve residue in 100 μL mobile phase, vortex vigorously, inject 50 μL aliquot.

HPLC VARIABLES

Guard column: pellicular cyano

Column: 150 × 4.6 5 μm Zorbax CN

Mobile phase: MeCN:50 mM KH₂PO₄ 17:83 containing 5 mM sodium octanesulfonate, pH 4.9

Flow rate: 1.2
Injection volume: 50
Detector: F ex 285 em 345

CHROMATOGRAM

Retention time: 5.7
Internal standard: isopropylmorphine (9)
Limit of detection: 5 ng/mL

KEY WORDS

plasma

REFERENCE

Mohammed, S.S.; Butschkau, M.; Derendorf, H. A reversed phase liquid chromatographic method for the determination of codeine in biological fluids with applications. *J.Liq.Chromatogr.*, **1993**, *16*, 2325–2334

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 40 μ m bonded silica Clean Screen SPE cartridge (Worldwide Monitoring) with 3 mL MeOH, 3 mL water, and 1 mL pH 3 phosphate buffer. 1 mL Plasma + 2 mL 10 mM phosphoric acid, mix, add to the SPE cartridge, air dry for 30 s, wash with 1 mL pH 3 phosphate buffer, wash with 1 mL MeOH, air dry for 30 s, elute with 3 mL 2% ammoniacal MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 200 \times 4.5 5 μ m LiChrosphere diol
Mobile phase: MeCN:50 mM NaH₂PO₄ 80:20 pH, adjusted to 3 with orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 6
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, glucuronides, morphine, normorphine

KEY WORDS

plasma; SPE

REFERENCE

Wielbo, D.; Bhat, R.; Chari, G.; Vidyasagar, D.; Tebbett, I.R.; Gulati, A. High-performance liquid chromatographic determination of morphine and its metabolites in plasma using diode-array detection. *J.Chromatogr.*, **1993**, *615*, 164–168

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 1 M phosphoric acid + 5 mL butyl chloride, mix for 1.5 min, centrifuge at 1500 g for 3 min, discard upper organic layer. To the aqueous layer add 500 μ L pH 10 1 M carbonate buffer and 5 mL butyl chloride, mix for 1.5 min, centrifuge at 1500 g for 3 min, remove organic layer and repeat extraction. Combine butyl chloride layers and evaporate them to dryness under a stream of air at 40°. Reconstitute the residue in 200 μ L mobile phase, inject a 150 μ L aliquot.

HPLC VARIABLES**Guard column:** Novapak C18 guard column**Column:** 4 μm Novapak C18 in a Waters RCM 8 \times 10 radial compression unit**Mobile phase:** MeOH:MeCN:10 mM pH 7 phosphate buffer 230:20:1000, containing 40 mg/L cetyltrimethylammonium bromide (cetavlon)**Flow rate:** 2**Injection volume:** 150**Detector:** E, Waters Model 460, working electrode 1.10 V

CHROMATOGRAM**Retention time:** 13.5**Internal standard:** codeine

OTHER SUBSTANCES**Simultaneous:** oxycodone**Noninterfering:** acetaminophen, amitriptyline, aspirin, atenolol, camazepam, carbamazepine, chlorimipramine, chlorthalidone, clonazepam, cortisone, desipramine, diazepam, halazepam, hydrochlorothiazide, hydrocortisone, imipramine, lorazepam, maprotiline, meperidine, methylphenobarbital, methylprednisolone, metoclopramide, midazolam, morphine, nalorphine, naloxone, nitrazepam, nortriptyline, oxazepam, oxprenolol, phenobarbital, phenytoin, pindolol, prazepam, prednisolone, prednisone, primidone, prochlorperazine, propranolol, salicylic acid, temazepam

KEY WORDS

plasma; codeine is IS

REFERENCESmith, M.T.; Watt, J.A.; Mapp, G.P.; Cramond, T. Quantitation of oxycodone in human plasma using high-performance liquid chromatography with electrochemical detection. *Ther. Drug Monit.*, **1991**, *13*, 126-130

SAMPLE**Matrix:** blood, CSF**Sample preparation:** Prepare 500 mg 3 mL Bond Elut C2 SPE 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 cartridge and wash 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.

HPLC VARIABLES**Guard column:** hexyl**Column:** 150 \times 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:** 13**Limit of detection:** 1.11 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites, dihydrocodeine, morphine, normorphine**Simultaneous:** diamorphine, dihydrocodeine

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: Condition two 130 mg Sep-Pak Light C18 SPE cartridges with 1 mL MeOH and 1 mL water. Dilute urine, if necessary, 20-fold with water. 1 mL Plasma, urine, or diluted urine + 1 mL 500 mM pH 9.3 ammonium sulfate buffer, mix, add 1.9 mL of this mixture to a SPE cartridge at 0.75 mL/min, wash with 4 mL 5 mM pH 9.1 ammonium sulfate buffer at 1.5 mL/min, wash with 200 μ L MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min, elute with 600 μ L MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min. Mix the eluate with 1 mL 500 mM pH 9.3 ammonium sulfate buffer, add to a second SPE cartridge at 0.75 mL/min, wash with 4 mL 5 mM pH 9.1 ammonium sulfate buffer at 1.5 mL/min, wash with 200 μ L MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min, elute with 600 μ L MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min, inject a 400 μ L aliquot of the eluate.

HPLC VARIABLES

Column: 100 \times 4 3 μ m Spherisorb S3 ODS2

Mobile phase: MeCN:buffer 22:78 (Buffer was 30 mM KH_2PO_4 containing 3 mM dodecyl sulfate, adjusted to pH 2.1 with phosphoric acid.)

Flow rate: 1.5

Injection volume: 400

Detector: UV 214

CHROMATOGRAM

Retention time: 22

Limit of detection: 20 nM

OTHER SUBSTANCES

Extracted: metabolites, morphine (electrochemical detection), norcodeine, normorphine (electrochemical detection)

KEY WORDS

plasma; SPE

REFERENCE

Svensson, J.O.; Yue, Q.Y.; Säwe, J. Determination of codeine and metabolites in plasma and urine using ion-pair high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 674, 49–55

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a Toxiclean SPE cartridge (Alltech) with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μ L Plasma or serum + 100 μ L MeOH + 200 μ L MeCN + 100 μ L 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 μ L 2.5 μ g/mL flufenamic acid 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 μ L Urine + 100 μ L MeOH + 200 μ L MeCN + 500 μ L 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 μ L 2.5 μ g/mL flufenamic acid 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 \times 4.6 5 μ m Adsorbosphere HS C18

Mobile phase: MeCN:MeOH:1.2% ammonium acetate 15:40:45

Flow rate: 0.8

Detector: UV 239

CHROMATOGRAM

Retention time: 9.17

Internal standard: flufenamic acid (24.39)

Limit of quantitation: 100 ng/mL (blood); 300 ng/mL (urine)

OTHER SUBSTANCES

Extracted: monoacetylmorphine, morphine, papaverine

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: bulk

Sample preparation: Prepare a 750 μ g/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μ m), inject a 15 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m LiChrospher 100

Column: 125 \times 4 3 μ m 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: 6.7

OTHER SUBSTANCES

Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, 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:** bulk**Sample preparation:** Dissolve in mobile phase, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μm μ Bondapak phenyl**Mobile phase:** MeOH:7 mM pH 3.1 triethylammonium phosphate buffer 20:80**Flow rate:** 1**Injection volume:** 25**Detector:** UV 254

CHROMATOGRAM**Retention time:** 7

OTHER SUBSTANCES**Simultaneous:** α -codeimethine, O⁶-codeine methyl ether, meconic acid, morphine

REFERENCE

Ayyangar, N.R.; Bhide, S.R.; Kalkote, U.R. Assay of semi-synthetic codeine base with simultaneous determination of alpha-codeimethine and O6-codeine methyl ether as by-product impurities by high-performance liquid chromatography. *J.Chromatogr.*, **1990**, 519, 250–255

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute syrup with mobile phase to a concentration of 5-100 $\mu\text{g/mL}$, shake, filter, inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μm 80 \AA 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:** 9.0

OTHER SUBSTANCES**Simultaneous:** bromhexine, chlorpheniramine, 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

HPLC VARIABLES**Column:** 300 \times 3.9 μ Bondapak 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: 4.9

OTHER SUBSTANCES

Simultaneous: acetaminophen, p-aminophenol, benzoic acid, 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: Dissolve capsules and tablets in MeOH:pH 4.0 water 1:1, shake for 1 (capsules) or 4 (tablets) h, dilute a 10 mL aliquot with 40 mL pH 3.2 water, filter (0.45 μm), collect last portion of filtrate, inject a 20 μL aliquot. (pH 3.2 and 4.0 water are prepared by adjusting pH of distilled water with phosphoric acid.)

HPLC VARIABLES

Column: 300 \times 3.9 $\mu\text{Bondapak C18}$

Mobile phase: MeOH:buffer 7:93 (Buffer was 15 mM KH_2PO_4 + 2 mL triethylamine per liter. Adjusted to pH 2.35 ± 0.1 with concentrated phosphoric acid.)

Column temperature: 40

Flow rate: 3

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Simultaneous: degradation products, acetaminophen, p-aminophenol, codeine-N-oxide, codeinone

KEY WORDS

capsules; tablets; rugged

REFERENCE

Sisco, W.R.; Rittenhouse, C.T.; Everhart, L.A. Simultaneous high-performance liquid chromatographic stability-indicating analysis of acetaminophen and codeine phosphate in tablets and capsules. *J.Chromatogr.*, **1985**, *348*, 253-263

SAMPLE

Matrix: formulations

Sample preparation: Add 1 tablet to 95 mL water, place on a steam bath for 15 min, cool, mix for 15 min, sonicate, allow to stand, filter, inject 13 μ L aliquot.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeOH:buffer 25:75 (Buffer was 0.01 N KH_2PO_4 + 50 mM KNO_3 , adjusted to pH 4.5 with 3 N phosphoric acid.)

Flow rate: 1.1

Injection volume: 13

Detector: UV 283

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: acetaminophen, p-aminophenol, p-chloroacetanilide, hydrocodone, hydro-morphone

KEY WORDS

tablets

REFERENCE

Wallo, W.E.; D'Adamo, A. Simultaneous assay of hydrocodone bitartrate and acetaminophen in a tablet formulation. *J.Pharm.Sci.*, **1982**, *71*, 1115-1118

SAMPLE

Matrix: microsomal incubations

Sample preparation: Prepare a 1 mL 100 mg C18 Bond Elut SPE cartridge by washing with 1 mL MeOH, 1 mL water, 1 mL 5 mM pH 9.0 carbonate buffer. Mix 100 μ L microsomal incubation, 20 μ L 25 $\mu\text{g}/\text{mL}$ 10,11-dihydrocarbamazepine in MeCN:water 25:75, 600 μ L 200 mM pH 10.2 carbonate buffer, 80 μ L 20 mM tetrabutylammonium hydrogen sulfate in water with vortex mixing after each addition. Add to the SPE cartridge, wash with 1 mL 5 mM pH 9.0 carbonate buffer, elute with 0.5 mL MeCN:mobile phase buffer 40:60.

HPLC VARIABLES

Guard column: 20 \times 2 Phase Separations pellicular ODS

Column: 250 \times 4.6 5 μm Hypersil CPS (cyanopropyl)

Mobile phase: MeCN:buffer 24:76 (Buffer was 50 mM potassium hydrogen phosphate containing 1 mM sodium dodecyl sulfate adjusted to pH 2.5 with orthophosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 210; E, ESA Coulochem II with a 5020 guard cell (+0.60 V) and a 5011 analytical cell (cell 1 +0.22 V, cell 2 +0.45 V)

CHROMATOGRAM

Retention time: 14

Internal standard: 10,11-dihydrocarbamazepine

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, morphine, norcodeine

KEY WORDS

SPE

REFERENCE

Pawula, M.; Shaw, P.N.; Barrett, D.A. Determination of codeine and its metabolites in microsomal incubates by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *653*, 106–111

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.1 6 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: 2

OTHER SUBSTANCES

Simultaneous: atropine, barbituric acid, diphenhydramine, 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: 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: 6.09 (A), 3.42 (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, 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, flvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydroalazine, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, nicllobemide, 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

some 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 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, bromazepam, 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, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cyamarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyron, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, 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, im-

inostilbene, imipramine, indomethacin, isocarboxtyril, 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, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naprofen, nefopam, niacinamide, nicotine, nicotinic acid, 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, pyrillamine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, 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, tripeleennamine, triproilidine, 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 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: 5

OTHER SUBSTANCES

Simultaneous: cocaine, 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: 2.016

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, 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: Inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelco C18
Mobile phase: MeCN:buffer 70:30 (Buffer contained 2.88% sodium lauryl sulfate and 1.248% NaH_2PO_4 , adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

Simultaneous: drotaverine, ethaverine, moxaverine, papaverine

REFERENCE

Girgis, E.H. Ion-pair reversed-phase liquid chromatographic identification and quantitation of papaverine congeners. *J.Pharm.Sci.*, **1993**, 82, 503–505

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m Applied Biosystems pre-column
Column: 100 \times 2 10 μ m μ Porasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 16.0
Limit of detection: 2.9 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, butorphanol, ethylmorphine, fentanyl, meperidine, morphine, nalbuphine, nalorphine, tramadol

Noninterfering: atropine, diazepam, neostigmine, pancuronium, succinylcholine, thiopental

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: 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: 15

OTHER SUBSTANCES

Simultaneous: atropine, dansylamide, dansylcadaverine, doxorubicin, 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: solutions

HPLC VARIABLES

Column: Supelco LC-8

Mobile phase: MeOH:water:acetic acid 40:59:1 containing 100 mM potassium nitrate, 10 mM tetramethylammonium bromide, and 2.5 mM heptanesulfonic acid

Flow rate: 1

Detector: E, Metrohm 1096/2, platinum working electrode +0.4 V, Ag/AgCl reference electrode following post-column reaction. The column effluent passed through an electrochemical cell (construction details in paper) and the bromide was oxidized to bromine at 3 μA. The mixture flowed through a 20 s reaction coil (3.9 m (?) × 0.33 mm ID) to the detector.

CHROMATOGRAM

Retention time: 5

Limit of detection: 0.5 ng

OTHER SUBSTANCES

Simultaneous: morphine, noscapine, papaverine

KEY WORDS

post-column reaction

REFERENCE

Kok, W.T.; Brinkman, U.A.T.; Frei, R.W. On-line electrochemical reagent production for detection in liquid chromatography and continuous flow systems. *Anal.Chim.Acta*, **1984**, *162*, 19-32

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 2.92

OTHER SUBSTANCES

Simultaneous: acetylcodeine, amphetamine, benzphetamine, benzylmorphine, buprenorphine, caffeine, chlorphentermine, dextromoramide, dextropropoxyphene, diamorphine, diethylpropion, dihydrocodeine, dihydromorphine, dimethylamphetamine, dipipanone, ephedrine, epinephrine, ethoheptazine, etorphine, fencamfamin, fenethyline, fenfluramine, fentanyl, hydrocodone, hydroxypethidine, levallorphan, levorphanol, mazindol, meperidine, mephentermine, mescaline, methadone, methamphetamine, methylenedioxymphetamine, methylephedrine, methylphenidate, monoacetylmorphine, morphine-3-glucuronide, nalorphine, naloxone, norcodeine, norlevorphanol, normethadone, normorphine, norpethidine, norpipanone, norpseudoephedrine, noscapine, oxycodone, papaverine, pemoline, pentazocine, phenazocine, phendimetrazine, phenelzine, phenoperidine, phentermine, phenylephrine, phenylpropanolamine, pholcodeine, pipradol, piritramide, pseudoephedrine, thebacon, thebaine, tranlycypromine, trimethoxyamphetamine, tyramine

Noninterfering: dopamine, levodopa, methyl dopa, methyl dopate, norepinephrine

Interfering: bromo-STP, codeine-N-oxide, ethylmorphine, 4-hydroxyamphetamine, morphine, morphine-N-oxide, normetanephrine, 2-phenethylamine, prolintane, STP

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 hydrolyzed 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 unhydrolyzed urine to determine diamorphine and unconjugated compounds.)

HPLC VARIABLES

Column: 200 \times 2.3 μ m 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: 8

Internal standard: nalorphine (5)

Limit of detection: <20 ng/mL

OTHER SUBSTANCES

Extracted: diamorphine, 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

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μ m), inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 Lichrospher 5 μ m 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: 4.5

OTHER SUBSTANCES

Extracted: amitriptyline, amphetamine, benzoylecgonine, meperidine, morphine, nordiazepam, norpropoxyphene

Also analyzed: cocaine (different gradient), diphenhydramine, lidocaine, nortriptyline, phenylpropanolamine

Interfering: ephedrine

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: 1 mL Urine + 10 μ L nalorphine solution + 3000-3500 U glucuronidase (*Patella vulgata*, Sigma) + 300 μ L 1.1 M pH 5 sodium acetate buffer, heat overnight at 55°, add 500 μ L buffer, add 8 mL chloroform:isopropanol 90:10, rotate gently for 30 min, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 3 mL pH 9.9 NaH_2PO_4 buffer, rotate gently for 10 min, centrifuge, discard the aqueous layer, repeat the wash. Remove the organic layer and add it to 200 μ L 0.2% phosphoric acid, rotate gently for 30 min, inject a 50 μ L aliquot of the aqueous layer. (Buffer was 50 mM boric acid and 43 mM sodium tetraborate, pH adjusted to 9.9.)

HPLC VARIABLES

Guard column: Nova-Pak phenyl

Column: 150 \times 3.9 5 μ m Nova-Pak phenyl

Mobile phase: MeCN:10 mM pH 6.6 NaH_2PO_4 10:90

Flow rate: 1.2

Injection volume: 50

Detector: E, ESA Coulochem, Model 5010 analytical cell, detector cell 1 +0.20 V, detector cell 2 + 0.55 V, model 5020 guard cell + 0.75 V; UV 210

CHROMATOGRAM

Retention time: 19.2

Internal standard: nalorphine (25.2)

Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: 6-monoacetylmorphine, morphine

Simultaneous: dihydrocodone, hydrocodone, oxycodone

Noninterfering: 7-aminoclonazepam, 7-aminoflunitrazepam, amitriptyline, amphetamine, diazepam, dothiepin, doxepin, ephedrine, mesoridazine, methadone, methamphetamine, nordiazepam, norpropoxyphene, nortriptyline, oxazepam, propoxyphene, quinidine, quinine, sulfamethoxazole, sulforidazine, thioridazine, trimethoprim

REFERENCE

Gerostamoulos, J.; Crump, K.; McIntyre, I.M.; Drummer, O.H. Simultaneous determination of 6-monoacetylmorphine, morphine and codeine in urine using high-performance liquid chromatography with combined ultraviolet and electrochemical detection. *J.Chromatogr.*, **1993**, 617, 152-156

SAMPLE

Matrix: urine

Sample preparation: 10 mL Urine + 500 μ L 100 μ g/mL nalorphine hydrobromide in MeOH + 1 mL concentrated HCl, heat at 100° for 1 h, cool, add 500 μ L saturated ammonium sulfate solution, adjust pH to 9 with 25% NaOH, dilute to 20 mL with water, add mixture to an Extrelut 20 column, let stand for 10 min, elute with 40 mL dichloromethane:isopropanol 85:15. Add the eluate to 3 mL 200 mM HCl, extract, repeat extraction. Combine the aqueous phases and add them to 500 μ L saturated ammonium sulfate solution, adjust pH to 9.2 with 25% NaOH, dilute to 20 mL with water, add to another Extrelut 20 column, let stand for 10 min, elute with 40 mL dichloromethane:isopropanol 85:15. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m Lichrosorb

Column: 250 \times 4 5 μ m Lichrospher Si 100

Mobile phase: n-Hexane:dichloromethane:MeOH containing 0.75% diethylamine 72.5:20:7.5

Flow rate: 1.35

Injection volume: 20

Detector: UV 225

CHROMATOGRAM**Retention time:** 8.3**Internal standard:** nalorphine (6)

OTHER SUBSTANCES**Extracted:** morphine**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; normal phase

REFERENCEFerrara, 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:** 500 μ L Urine + N-ethylnordiazepam + 100 μ L buffer, centrifuge at 11000 g for 30 s, inject a 500 μ L 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 μ L mobile phase B, with 200 μ L 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 start 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 \times 2.1 12-20 μ m PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 \times 3.2 11 μ m Aminex A-28 (Bio-Rad); C 25 \times 3.2 5 μ m C8 (Phenomenex) + 150 \times 4.6 5 μ m 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:** 40 (B, C only)**Flow rate:** A 5; B-E 1**Injection volume:** 500**Detector:** UV 210; UV 235

CHROMATOGRAM**Retention time:** k' 5.7**Internal standard:** N-ethylnordiazepam (k' 2.1)**Limit of detection:** 300 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, amphetamine, benzoylecgonine, caffeine, cotinine, desipramine, diazepam, diphenhydramine, ephedrine, flurazepam, hydrocodone, hydromorphone, imipramine, lidocaine, methadone, methamphetamine, morphine, nordiazepam, nortriptyline, oxazepam, pentazocine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, secobarbital

Interfering: chlorpheniramine

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

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Theodoridis, G.; Papadoyannis, I.; Vasilikiotis, G.; Tsoukali-Papadopoulou, H. Reversed-phase high-performance liquid chromatography–photodiode-array analysis of alkaloid drugs of forensic interest. *J.Chromatogr.B*, **1995**, *668*, 253–263 [also amphetamine, bamifylline, caffeine, cocaine, diamorphine, ethylmorphine, flufenamic acid, hyoscyamine, methadone, morphine, nalorphine, norcodeine, papaverine, quinine, scopolamine, strychnine, theobromine, theophylline, tolfenamic acid]

Band, C.J.; Band, P.R.; Deschamps, M.; Besner, J.-G.; Coldman, A.J. Human pharmacokinetic study of immediate-release (codeine phosphate) and sustained-release (codeine contin) codeine. *J.Clin.Pharmacol.*, **1994**, *34*, 938–943 [electrochemical detection; SPE; plasma; ethylmorphine (IS)]

Papadoyannis, I.; Zotou, A.; Samanidou, V.; Theodoridis, G.; Zougrou, F. Comparative study of different solid-phase extraction cartridges in the simultaneous RP-HPLC analysis of morphine and codeine in biological fluids. *J.Liq.Chromatogr.*, **1993**, *16*, 3017–3040 [simultaneous caffeine, morphine, quinine, strychnine; SPE; urine; plasma; LOD 10-20 ng/mL]

Berthod, A.; Laserna, J.J.; Carretero, I. Oil-in-water microemulsions as mobile phases for rapid screening of illegal drugs in sports. *J.Liq.Chromatogr.*, **1992**, *15*, 3115–3127 [also acebutolol, chlorthalidone, hydrochlorothiazide, methoxamine, methyltestosterone, nadolol, norcodeine, oxprenolol, phenylephrine, probenecid]

Heybroek, W.M.; Caulfield, M.; Johnston, A.; Turner, P. Automatic on-line extraction coupled with electrochemical detection as an improved method for the HPLC co-analysis of codeine and morphine in plasma and gastric juice. *J.Pharm.Biomed.Anal.*, **1990**, *8*, 1021–1027

Chen, Z.R.; Bochner, F.; Somogyi, A. Simultaneous determination of codeine, norcodeine and morphine in biological fluids by high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1989**, *491*, 367–378

Persson, K.; Lindstrom, B.; Spalding, D.; Wahlstrom, A.; Rane, A. Determination of codeine and its metabolites in human blood plasma and in microsomal incubates by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1989**, *491*, 473–480

Harris, S.C.; Miller, M.A.; Wallace, J.E. Determination of codeine and morphine in human plasma by high performance liquid chromatography with serial electrochemical detection. *Ann.Clin.Lab.Sci.*, **1988**, *18*, 297–305 [also ethylmorphine, morphine, nalorphine; electrochemical detection]

Janicot, J.L.; Caude, M.; Rosset, R. Separation of opium alkaloids by carbon dioxide sub- and supercritical fluid chromatography with packed columns. Application to the quantitative analysis of poppy straw extracts. *J.Chromatogr.*, **1988**, *437*, 351–364 [SFC; simultaneous cryptopine, morphine, narcotine, papaverine, thebaine]

Alvi, S.U.; Castro, F. A stability-indicating simultaneous analysis of acetaminophen and hydrocodone bitartrate in tablets formulation by HPLC. *J.Liq.Chromatogr.*, **1987**, *10*, 3413–3426 [stability-indicating; simultaneous acetaminophen, hydrocodone, hydromorphone; tablets; column temp 30]

Gibson, M.; Jefferies, T.M.; Soper, C.J. Isolation of codeine and norcodeine from microbial transformation liquors by preparative high-performance liquid chromatography. *Analyst*, **1987**, *112*, 1667–1670

- Lurie, I.S.; McGuinness, K. The quantitation of heroin and selected basic impurities via reversed phase HPLC. II. The analysis of adulterated samples. *J.Liq.Chromatogr.*, **1987**, *10*, 2189–2204 [UV detection; electrochemical detection also acetaminophen, acetylcodeine, acetylmorphine, acetylprocaine, aminopyrene, amitriptyline, antipyrine, aspirin, barbital, benztropine, caffeine, cocaine, diamorphine, diazepam, diphenhydramine, dipyrone, ephedrine, ethylmorphine, lidocaine, meconin, methamphetamine, methapyrilene, methaqualone, morphine, nalorphine, niacinamide, noscapine, papaverine, phenacetin, phenmetrazine, phenobarbital, phenolphthalein, procaine, propanophenone, propoxyphene, pyrilamine, quinidine, quinine, salicylamide, salicylic acid, secobarbital, strychnine, tartaric acid, tetracaine, thebaine, tripeleminamine, tropacocaine, vitamin B3, vitamin B5]
- Huttner, A.; Eigendorf, H.G. [Simultaneous determination of propyphenazone, caffeine and codeine in drug mixtures by reverse phase HPLC]. *Pharmazie*, **1986**, *41*, 59
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- Meinsma, D.A.; Radzik, D.M.; Kissinger, P.T. Determination of common analgesics in serum and urine by liquid chromatography/electrochemistry. *J.Liq.Chromatogr.*, **1983**, *6*, 2311–2335 [serum; urine; electrochemical detection; extracted methyl salicylate, naproxen, phenacetin, salicylic acid]
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- Lurie, I.S.; Demchuk, S.M. Optimization of a reverse phase ion-pair chromatographic separation for drugs of forensic interest. II. Factors effecting selectivity. *J.Liq.Chromatogr.*, **1981**, *4*, 357–374 [also acetaminophen, acetylcodeine, acetylmorphine, aminopyrene, aminopyrine, amobarbital, amphetamine, antipyrine, benzocaine, butabarbital, caffeine, cocaine, diamorphine, diazepam, diethylpropion, DMT, ephedrine, glutethimide, Lampa, lidocaine, LSD, MDA, mecloqualone, mescaline, methamphetamine, methapyrilene, methaqualone, methpyrilene, methylphenidate, morphine, narcotine, papaverine, PCP, pentobarbital, phencyclidine, phendimetrazine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, procaine, quinidine, quinine, secobarbital, strychnine, TCP, tetracaine, thebaine, theophylline]
- Lurie, I.S.; Demchuk, S.M. Optimization of a reverse phase ion-pair chromatographic separation for drugs of forensic interest. I. Variables affecting capacity factors. *J.Liq.Chromatogr.*, **1981**, *4*, 337–355 [acetylcodeine, acetylmorphine, aminopyrene, aminopyrine, amobarbital, antipyrine, butabarbital, diamorphine, methapyrilene, morphine, narcotine, papaverine, pentobarbital, phenobarbital, quinidine, quinine, secobarbital, strychnine, thebaine]
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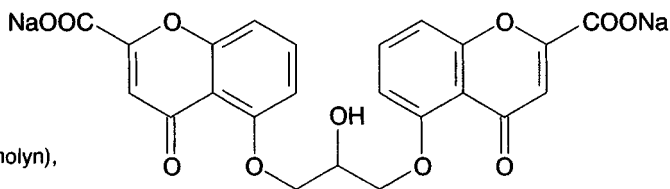
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- Kubiak, E.J.; Munson, J.W. High-performance liquid chromatographic analysis of codeine in syrups using ion-pair formation. *J.Pharm.Sci.*, **1980**, *69*, 152–156 [syrup; simultaneous ethylmorphine, morphine]
- Muhammad, N.; Bodnar, J.A. Quantitative determination of guaifenesin, phenylpropanolamine hydrochloride, sodium benzoate & codeine phosphate in cough syrups by high-pressure liquid chromatography. *J.Liq.Chromatogr.*, **1980**, *3*, 113–122
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Cromolyn

Molecular formula: C₂₃H₁₆O₁₁

Molecular weight: 468.4

CAS Registry No.: 16110-51-3 (cromolyn),
15826-37-6 (cromolyn sodium)



SAMPLE

Matrix: bile, blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 1 mL water + 200 μ L concentrated HCl + 3 mL ethyl acetate, shake vigorously for 10 min, centrifuge at 3000 rpm for 10 min. Remove a 2.5 mL aliquot of the organic layer and add it to 100 μ L 100 mM pH 7.0 phosphate buffer, shake vigorously for 10 min, centrifuge at 3000 rpm for 10 min, inject a 30 μ L aliquot of the aqueous layer. Urine, bile. 5 mL Urine or bile + 500 μ L concentrated HCl, mix, add 5 mL ethyl acetate, shake vigorously for 10 min, centrifuge at 3000 rpm for 10 min. Remove a 4 mL aliquot of the organic layer and add it to 500 μ L 100 mM pH 7.0 phosphate buffer, shake vigorously for 10 min, centrifuge at 3000 rpm for 10 min, inject a 15 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 200 \times 4 10 μ m Nucleosil NH₂

Mobile phase: MeCN:68 mM pH 3.0 KH₂PO₄/phosphoric acid 35:65 (rat) or MeCN:78 mM pH 2.5 KH₂PO₄/phosphoric acid 35:65 (rabbit)

Flow rate: 2

Injection volume: 15-30

Detector: UV 240

KEY WORDS

plasma; rat; rabbit; pharmacokinetics

REFERENCE

Yoshimi, A.; Hashizume, H.; Kitagawa, M.; Nishimura, K.; Kakeya, N. Characteristics of 1,3-bis-(2-ethoxycarbonylchromon-5-yloxy)-2-((S)-lysyloxy)propane dihydrochloride (N-556), a prodrug for the oral delivery of disodium cromoglycate, in absorption and excretion in rats and rabbits. *J.Pharmacobiodyn.*, **1992**, *15*, 681-686

SAMPLE

Matrix: bulk

Sample preparation: Inject a 10 μ L aliquot of a solution in MeCN:water 50:50.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m Resolve octadecylsilane (Waters)

Mobile phase: MeCN:water 50:50 containing 0.5 g/L cetyltrimethylammonium bromide (Use a 100 \times 9.4 column of 8 μ m CSC-S silica between pump and injector.)

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 11.79

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Duhaime, R.M.; Rollins, L.K.; Gorecki, D.J.K.; Lovering, E.G. Liquid chromatographic determination of cromolyn sodium and related compounds in raw materials. *JAOAC Int.*, **1994**, *77*, 1439–1442

SAMPLE

Matrix: formulations

Sample preparation: Solutions, capsules. Dilute solutions and capsule contents with water so as to achieve a cromolyn concentration of 40 µg/mL, inject a 20 µL aliquot. Aerosols. Direct aerosol into a flask, dissolve collected sample in water so as to achieve a cromolyn concentration of 40 µg/mL, filter (0.45 µm), inject a 20 µL aliquot of the filtrate

HPLC VARIABLES

Column: 250 × 4.6 5 µm Nucleosil octadecylsilane

Mobile phase: MeCN:1% phosphoric acid 25:75

Flow rate: 1.5

Injection volume: 20

Detector: UV 238

CHROMATOGRAM

Retention time: 5.15

KEY WORDS

inhalation solution; nasal solution; capsules; aerosols

REFERENCE

Ng, L.L. Reversed-phase liquid chromatographic determination of cromolyn sodium in drug substances and select dosage forms. *JAOAC Int.*, **1994**, *77*, 1689–1694

SAMPLE

Matrix: formulations

Sample preparation: Extract capsule contents or gels with water, filter (0.45 µm), inject a 10 µL aliquot. Dilute drops to 0.39 mM, filter (0.45 µm), inject a 10 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 µm C18 Nova-Pak

Mobile phase: MeOH:10 mM ammonium dihydrogen phosphate 50:50, pH adjusted to 2.3 with orthophosphoric acid

Flow rate: 1

Injection volume: 10

Detector: UV 326

CHROMATOGRAM

Retention time: 2.65

KEY WORDS

capsules; gels; drops

REFERENCE

Radulovic, D.; Kocic-Pesic, V.; Pecanac, D.; Zivanovic, L. HPLC determination of sodium cromoglycate in pharmaceutical dosage forms. *Farmaco*, **1994**, *49*, 375–376

SAMPLE

Matrix: solutions

HPLC VARIABLES**Column:** 250 × 4.6 Zorbax SAX**Mobile phase:** MeOH:buffer 50:50 (Buffer was 180 mM Na₂HPO₄ adjusted to pH 3.00 ± 0.05 with 180 mM orthophosphoric acid. Pass mobile phase through a 250 × 4.6 25-40 μm silica (HPLC Technology) column to saturate it with silica.)**Flow rate:** 1**Detector:** UV 253

CHROMATOGRAM**Retention time:** 9.5

OTHER SUBSTANCES**Simultaneous:** acetaminophen, albuterol, aspartame, aspirin, beclomethasone dipropionate, caffeine, isoproterenol, menthol, minocromil, nedocromil, quinoline yellow, reproterol, riboflavin, saccharin, salicylic acid, sorbitan trioleate, terbutaline, theophylline

REFERENCEBaker, P.R.; Gardner, J.J.; Wilkinson, D. Automated high-performance liquid chromatographic method for the determination of nedocromil sodium in human urine using bimodal column switching. *J.Chromatogr.B*, 1995, 668, 59-65

SAMPLE**Matrix:** urine**Sample preparation:** 10 mL Urine + 5 g NaCl + 1 mL water + 1 mL concentrated HCl + 10 mL diethyl ether, shake for 10 min at 200 oscillations/min, centrifuge at 1540 g for 10 min. Repeat extraction. Combine extracts, add 1 mL 1 M pH 3.5 glycine HCl buffer, shake, centrifuge, inject aliquot of lower aqueous phase

HPLC VARIABLES**Column:** 250 × 4.6 10 μm Partisil SAX**Mobile phase:** 0.9 M Orthophosphoric acid adjusted to pH 2.30 ± 0.01 with 5 M NaOH**Flow rate:** 3.6**Injection volume:** 120**Detector:** UV 325

CHROMATOGRAM**Retention time:** 4.5**Limit of detection:** 50 ng/mL

OTHER SUBSTANCES**Noninterfering:** acetaminophen, aspirin, hydrocortisone, phenylbutazone, prednisolone, salicylic acid, terbutaline, theophylline

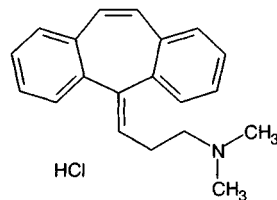
REFERENCEGardner, J.J. Determination of sodium cromoglycate in human urine by high-performance liquid chromatography on an anion-exchange column. *J.Chromatogr.*, 1984, 305, 228-232

Cyclobenzaprine

Molecular formula: C₂₀H₂₁N

Molecular weight: 275.4

CAS Registry No.: 303-53-7 (cyclobenzaprine), 6202-23-9
(cyclobenzaprine hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 500 μ L 250 μ g/mL protriptyline hydrochloride + 1 mL 500 mM NaOH + 4 mL toluene:n-hexane:isoamyl alcohol 77:22:3, mix for 10 min, centrifuge at 3000 rpm for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μ L MeOH, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.7 5 μ m Supelcosil LC-PCN cyanopropyl

Mobile phase: MeCN:MeOH:10 mM pH 7.2 potassium phosphate buffer 60:15:25 (Prepare buffer by mixing 194 mL 1.36 g/L KH₂PO₄ with 274 mL 1.74 g/L K₂HPO₄.)

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 3.7

Internal standard: protriptyline (8.1)

OTHER SUBSTANCES

Extracted: norcyclobenzaprine, nortriptyline

Interfering: amitriptyline

KEY WORDS

serum

REFERENCE

Wong, E.C.C.; Koenig, J.; Turk, J. Potential interference of cyclobenzaprine and norcyclobenzaprine with HPLC measurement of amitriptyline and nortriptyline: resolution by GC-MS analysis. *J.Anal.Toxicol.*, **1995**, *19*, 218-224

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 500 μ L 10 μ g/mL nortriptyline hydrochloride in water + 500 μ L 100 mM HCl, mix, add 500 μ L saturated sodium bicarbonate, vortex briefly, when effervescence ceases add 10 mL dichloromethane:pentane 30:70, shake for 25 min, centrifuge at 4° at 1400 g for 25 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μ L mobile phase, inject the whole amount.

HPLC VARIABLES

Guard column: Resolve Si Guard-PAK (Waters)

Column: 300 \times 3.9 10 μ m μ Porasil

Mobile phase: MeCN:EtOH:tert-butylamine 10:90:0.025

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 7.5

Internal standard: nortriptyline (13.7)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, aspirin, caffeine, ibuprofen

KEY WORDS

plasma; normal phase; use silanized glassware

REFERENCE

Hwang, P.T.R.; Young, D.A.; Straughn, A.B.; Meyer, M.C. Quantitative determination of cyclobenzaprine in human plasma by high pressure liquid chromatography. *J.Liq.Chromatogr.*, **1993**, *16*, 1163–1171

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μ L 500 ng/mL trimipramine in MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μ L mobile phase, inject a 150 μ L aliquot. Urine. 1 mL Urine + 1 mL β -glucuronidase (7200 Fishman Units) in 20 mM pH 6.5 phosphate buffer, heat at 37° for 24 h, add 100 μ L 10 M NaOH, add 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μ L mobile phase, inject a 150 μ L aliquot.

HPLC VARIABLES

Guard column: 20 mm long C18 base-deactivated silica (BDS) (Keystone)

Column: 250 \times 4.6 5 μ m C18 base-deactivated silica (BDS) (Keystone)

Mobile phase: MeCN:buffer 50:50 (plasma) or 43:57 (urine) (Buffer was 0.085% phosphoric acid adjusted to pH 6.5 with triethylamine.)

Flow rate: 1

Injection volume: 150

Detector: UV 229

CHROMATOGRAM

Retention time: 7.8 (plasma), 9.6 (urine)

Internal standard: trimipramine (10.5 (plasma), 12.8 (urine))

Limit of quantitation: 0.5 ng/mL (plasma); 10 ng/mL (urine)

KEY WORDS

plasma

REFERENCE

Constanzer, M.; Chavez, C.; Matuszewski, B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine. *J.Chromatogr.B*, **1995**, *666*, 117–126

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μ L 500 ng/mL trimipramine in MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150 μ L mobile phase, inject a 75 μ L aliquot. Urine. 1 mL Urine + 1 mL β -glucuronidase (7200 Fishman Units) in 20 mM pH 6.5 phosphate buffer, heat at 37° for 24 h, add 100 μ L 10 M NaOH, add 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 C18 base-deactivated silica (BDS) (Keystone)

Column: 50 \times 4.6 5 μ m C18 base-deactivated silica (BDS) (Keystone)

Mobile phase: MeCN:water 90:10 containing 0.1% formic acid and 10 mM ammonium acetate

Flow rate: 1

Injection volume: 50-75

Detector: MS, PE Sciex API III, heated nebulized interface, corona discharge needle +4 μ A, nebulizer probe 500°, nebulizing gas was air at 2 L/min and 80 psi, curtain gas flow was nitrogen at 0.9 L/min, sampling orifice +45 V, dwell time 400 ms, interface heater 60°, electron multiplier -3.7 kV, collision gas was argon 355×10^{12} atoms/cm², first quadrupole filter admits m/z 276 (cyclobenzaprine) and 295 (trimipramine, collisional fragmentation at second filter, monitor m/z 215 (cyclobenzaprine) and 208 (trimipramine) at third quadrupole filter

CHROMATOGRAM

Retention time: 1.9

Internal standard: trimipramine (2.2)

Limit of quantitation: 0.1 ng/mL (plasma); 10 ng/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Constanzer, M.; Chavez, C.; Matuszewski, B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine. *J.Chromatogr.B*, **1995**, *666*, 117-126

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets (60 mesh), shake with 25 mL 25 mM sulfuric acid for 1 h, add MeOH to 85 mL, swirl, allow to cool to room temperature, make up to 100 mL. Remove a 10 mL aliquot, add 5 mL 1 mg/mL naphazoline hydrochloride in MeOH, make up to 100 mL, filter (0.45 μ m), inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax C8

Mobile phase: MeCN:buffer 75:25 (Buffer was 12 g KH₂PO₄ in 1800 mL water, adjust pH to 3.0 with 1:3 phosphoric acid, make up to 2 L.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 6.8

Internal standard: naphazoline hydrochloride

OTHER SUBSTANCES**Simultaneous:** desipramine**Interfering:** amitriptyline

KEY WORDS

tablets

REFERENCE

Heintz, M.L. Determination of cyclobenzaprine in tablets by high-performance liquid chromatography. *J.Pharm.Sci.*, **1982**, *71*, 656-658

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 × 2 Deltabond C8 (Keystone)**Mobile phase:** MeCN:2-butanone:50 mM pH 7.0 phosphate buffer 27:13:60**Flow rate:** 0.15**Injection volume:** 1

Detector: Chemiluminescence following post-column reaction. A 1 mM solution of Ru(2,2'-bipyridine)₃²⁺ in 50 mM sodium sulfate (continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)₃³⁺ 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 was pumped at 0.3 mL/min and mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

CHROMATOGRAM**Retention time:** 6**Limit of detection:** 0.1-1 µg/mL

OTHER SUBSTANCES**Simultaneous:** dicyclomine

KEY WORDS

post-column reaction

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

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:** 15.50 (A), 7.05 (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, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, 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, methyl-dopa, 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, pimizide, 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, sulfapyrazone, 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

some 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 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, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, 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, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxycorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropranolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrrolamine, pyrithidione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylecypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triproilidine, 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 mobile phase.

HPLC VARIABLES

Column: 250 × 4.6 Econosil C8

Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH₂PO₄ and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)

Injection volume: 20

Detector: UV 210

CHROMATOGRAM**Retention time:** 8.6**Limit of quantitation:** <1 µg/mL

OTHER SUBSTANCES**Simultaneous:** doxepin, desipramine, protriptyline, maprotiline**Also analyzed:** amitriptyline, amoxapine, carbamazepine, imipramine, nortriptyline

KEY WORDSUV 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

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Puopolo, P.R.; Flood, J.G. Detection of interference by cyclobenzaprine in liquid- chromatographic assays of tricyclic antidepressants. *Clin.Chem.*, **1987**, *33*, 819–820 [LOD 10 ng/mL]

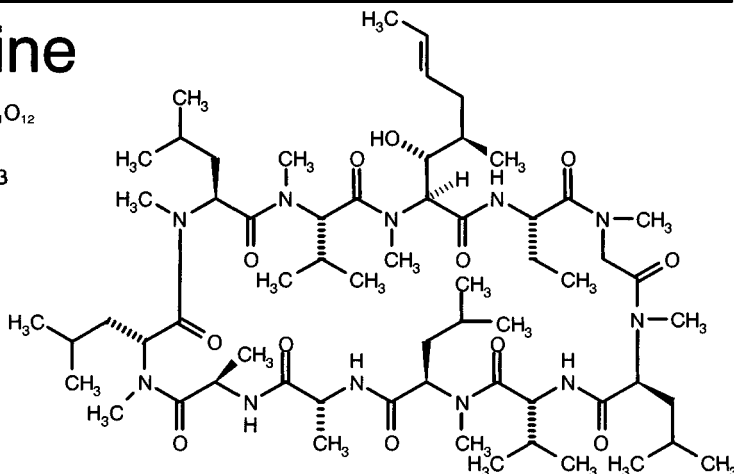
Schneider, M.; Giardina, E.-G.V. Interference by Flexeril, a tricyclic muscle relaxant, with liquid-chromatographic determination of imipramine. *Clin.Chem.*, **1986**, *32*, 1599 [plasma; interfering imipramine]

Cyclosporine

Molecular formula: C₆₂H₁₁₁N₁₁O₁₂

Molecular weight: 1202.6

CAS Registry No.: 59865-13-3



SAMPLE

Matrix: aqueous humor

Sample preparation: 10 Volumes aqueous humor + 1 volume 20 mg/mL cyclosporine D, mix, inject an aliquot.

HPLC VARIABLES

Guard column: Supelco guard column

Column: 250 × 4.6 5 μm C18 (Supelco)

Mobile phase: MeCN:water 70:30

Column temperature: 70

Flow rate: 1.5

Detector: UV 204

CHROMATOGRAM

Internal standard: cyclosporine D

Limit of quantitation: 250 ng/mL

KEY WORDS

rabbit; eye; pharmacokinetics

REFERENCE

Oh, C.; Saville, B.A.; Cheng, Y.-L.; Rootman, D.S. A compartmental model for the ocular pharmacokinetics of cyclosporine in rabbits. *Pharm.Res.*, **1995**, *12*, 433-437

SAMPLE

Matrix: bile, blood, feces, urine

Sample preparation: Whole blood. Add whole blood to 7-15 volumes MeOH, sonicate for 15 min, centrifuge at 6000 g for 20 min. Remove the supernatant and concentrate it under reduced pressure at 35°, inject an aliquot of the concentrate. Feces. Extract feces with 2 volumes MeOH, repeat extraction 3-4 times, combine the extracts, filter (5 μm), inject an aliquot. Urine, bile. Inject urine and bile directly.

HPLC VARIABLES

Column: two 150 × 4.6 Supelcosil LC-18 columns in series

Mobile phase: Gradient. A was MeOH:water 10:90. B was MeOH:MeCN 10:90. A:B from 100:0 to 55:45 over 29 min, to 45:55 over 60 min, to 10:90 over 32 min, maintain at 10:90 for 10 min.

Column temperature: 70

Flow rate: 1.5
Detector: UV 210

CHROMATOGRAM

Retention time: 109.7 (cyclosporin G)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

mouse; rat; dog; whole blood

REFERENCE

Mangold, J.B.; Rodriguez, L.C.; Wang, Y.K. Metabolism of cyclosporin G in the mouse, rat, and dog. *Drug Metab.Dispos.*, **1995**, *23*, 615–621

SAMPLE

Matrix: blood

Sample preparation: Serum + cyclosporine D, extract with 6 mL ethyl ether/petroleum ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 1 mL MeOH, wash the MeOH layer with 1.5 mL hexane/heptane. Evaporate the MeOH layer at 40°, reconstitute with mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 µm YWG

Mobile phase: MeOH:isopropanol:water 65:20:20

Column temperature: 65

Flow rate: 1

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Internal standard: cyclosporine D

Limit of detection: 25 ng/mL

Limit of quantitation: 50 ng/mL

KEY WORDS

serum

REFERENCE

Liang, M.Z.; Zou, Y.G.; Yu, Q.; Qing, Y.P. RPHPLC for monitoring blood concentration of cyclosporine A in kidney transplantation patient and clinical application (Abstract 65). *Ther.Drug Monit.*, **1995**, *17*, 399

SAMPLE

Matrix: blood

HPLC VARIABLES

Column: YWG-C18

Mobile phase: MeCN:water 80:22.5

Column temperature: 60

Flow rate: 1

Detector: UV 214

CHROMATOGRAM

Retention time: 6.8 (cyclosporine A)

Internal standard: cyclosporine D (9)

Limit of detection: 10 ng/mL

REFERENCE

Lin, S.G.; Yu, X.Y.; Yang, M. A modified HPLC method for measuring cyclosporine A and monitoring its blood concentration (Abstract 66). *Ther. Drug Monit.*, **1995**, *17*, 399

SAMPLE

Matrix: blood

Sample preparation: Condition a Varian C18 SPE cartridge with two 2 mL portions of MeCN and two 2 mL portions of MeCN:water 32:68. Condition a Varian silica SPE cartridge with two 2 mL portions of MeCN. 1 mL Whole blood + 400 μ L water + 3 mL cyclosporine C in MeCN:MeOH 90:10, vortex for 30 s, centrifuge at 3000 rpm for 10 min. Dilute the supernatant with 4.5 mL water, add to the C18 SPE cartridge, wash with 4 mL MeCN:water 32:68, wash with two 1.4 mL portions of MeOH:water 62:38, wash with 1.4 mL MeOH:300 mM acetic acid 60:40, wash with 3 mL hexane:acetone 99:1, elute with two 1.4 mL portions of MeCN:MeOH 75:25 through the silica SPE cartridge. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μ L mobile phase, add 300 μ L heptane, vortex for 30 s, centrifuge at 3000 rpm for 5 min, discard the heptane layer, repeat the heptane wash, inject an aliquot of the MeCN layer.

HPLC VARIABLES

Column: C8 (Beckman)

Mobile phase: MeCN:MeOH:water 51:20:9

Column temperature: 70

Flow rate: 0.6

Detector: UV 214

CHROMATOGRAM

Retention time: 38

Internal standard: cyclosporine C

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; SPE

REFERENCE

Liu, W.T.; Levy, G.A.; Wong, P.Y. Measurement of AM19 and other cyclosporine metabolites in the blood of liver transplant patients with stable liver function. *Ther. Drug Monit.*, **1995**, *17*, 479–486

SAMPLE

Matrix: blood

Sample preparation: 1 mL Whole blood + cyclosporine D in MeCN, centrifuge. Remove the supernatant and dilute it with water, add to a solid-phase extraction disk (15 mg SPEC RP 18 AR), wash with MeCN:water 20:80, wash with n-hexane, elute with MeCN. Evaporate the eluate to dryness, reconstitute, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 3 μ m nitrile

Mobile phase: n-Heptane:isopropanol 86:14

Column temperature: 54

Flow rate: 0.85
Detector: UV 210

CHROMATOGRAM

Retention time: 11.8 (cyclosporine A)
Internal standard: cyclosporine D (9.8)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; SPE

REFERENCE

Wenk, M.; Haefeli, W.E. Improved determination of cyclosporine and its metabolites using solid phase extraction disks and normal phase liquid chromatography (Abstract 72). *Ther. Drug Monit.*, **1995**, *17*, 401

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood + 4 mL MeCN:MeOH 9:1, mix, centrifuge, add the supernatant to an Analytichem 6 mL C18 SPE cartridge, wash with 3 mL MeOH: water 70:30, wash with 3 mL hexane:acetone 99:1, elute with 3 mL ethyl acetate:isopropanol 3:1. Pass the eluate through an Analytichem 3 mL silica SPE cartridge, collect the eluate and evaporate it to dryness, reconstitute the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 50 mm long LC-1 (Supelco)
Mobile phase: MeCN:water 46:54
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM

Retention time: 11.17
Internal standard: cyclosporin C (9.39)
Limit of detection: 25 ng/mL

KEY WORDS

whole blood; cyclosporin D, previously used as IS, is now unavailable; SPE

REFERENCE

Jones, P.M.; Brune, K. Monitoring cyclosporine by HPLC with cyclosporin C as internal standard. *Clin. Chem.*, **1993**, *39*, 168-169

SAMPLE

Matrix: blood

Sample preparation: Condition an AASP C8 SPE cartridge (Analytichem) with 1.5 mL isopropanol then with 1.5 mL MeCN:water 2:3, re-wet with a few drops of MeCN:water 2:3. 200 μ L Whole blood + 20 μ L 5 μ g/mL cyclosporin D in MeOH + 1.5 mL MeCN: water 2:3, vortex for 10 s, let stand for 20 min, centrifuge at 1300 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1.5 mL MeCN:water 2:3, purge with nitrogen at 1.4 bar for 5 min. Place SPE cartridge in a vacuum desiccator for 5 min, wash with 1 mL hexane, purge with nitrogen for 5 min. Purge with nitrogen for 3 min imme-

diately before injection, elute contents of SPE cartridge onto analytical column with mobile phase for 0.6 min.

HPLC VARIABLES

Column: 150 × 4.6 5 μm CPS Hypersil (cyanopropyl)

Mobile phase: Hexane:EtOH 91:9 (Place a 100 × 4.6 mm column packed with 37-53μm silica gel (Whatman Pre Column Gel) held at 53° between pump and SPE cartridge.)

Column temperature: 53

Flow rate: 0.7

Detector: UV 210

CHROMATOGRAM

Retention time: 7.2

Internal standard: cyclosporin D (8.2)

Limit of detection: 12.5 ng/mL

KEY WORDS

whole blood; SPE

REFERENCE

Lachno, D.R.; Patel, N.; Rose, M.L.; Yacoub, M.H. Improved high-performance liquid chromatographic method for analysis of cyclosporin A using an automated sample processor. *J.Chromatogr.*, **1990**, 525, 123-132

SAMPLE

Matrix: blood

Sample preparation: 0.5 mL Whole blood + 50 μL 5 μg/mL cyclosporin D, mix, add 2 mL 90 mM HCl, add 5 mL MTBE, rotate for 9 min, centrifuge at 500 g for 5 min. Remove the organic layer, add 2 mL 90 mM NaOH, shake vigorously for 3 min, centrifuge at 500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute in 60 μL mobile phase and 100 μL heptane, vortex, centrifuge, inject a 20 μL aliquot of the lower layer.

HPLC VARIABLES

Guard column: 15 mm long RP-8 guard column (Brownlee)

Column: 250 × 2 5 μm Astec microbore octyl (Advanced Separation Technologies)

Mobile phase: MeCN:MeOH:water 52:19:29

Column temperature: 70

Flow rate: 0.25

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 8.5

Internal standard: cyclosporin D (10)

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, N-acetylprocainamide, caffeine, carbamazepine, digoxin, ethosuximide, gentamicin, lidocaine, phenobarbital, phenytoin, primidone, procainamide, salicylic acid, theophylline, tobramycin, valproic acid

KEY WORDS

microbore; whole blood

REFERENCE

Annesley, T.; Matz, K.; Balogh, L.; Clayton, L.; Giacherio, D. Liquid-chromatographic analysis for cyclosporine with use of a microbore column and small sample volume. *Clin.Chem.*, **1986**, *32*, 1407-1409

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 25 μ L 25 μ g/mL cyclosporin D in MeOH, rinse pipette used for blood or plasma with 2 mL water and add the rinse to the mixture, add 14 mL ether, shake horizontally at 180 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove 11.5 mL of the organic phase and evaporate it to dryness, add 1 mL 25 mM HCl, add 2 mL MeOH, add 7 mL n-hexane, shake horizontally at 180 cycles/min for 5 min, centrifuge at 750 g for 5 min. Discard the n-hexane and wash the aqueous layer with another 7 mL n-hexane. Remove the aqueous phase and add it to 1 mL 25 mM NaOH and 7 mL ether, shake horizontally at 180 cycles/min for 10 min, centrifuge at 750 g for 5 min. Remove the ether layer and evaporate it to dryness, reconstitute with 100 μ L mobile phase, inject a 90 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m LC-18 (Supelco)

Mobile phase: MeCN:water 68.5:31.5

Column temperature: 75

Flow rate: 1.4

Injection volume: 90

Detector: UV 202

CHROMATOGRAM

Retention time: 5.77

Internal standard: cyclosporin D (7.82)

Limit of quantitation: 25 ng/mL

KEY WORDS

whole blood; plasma

REFERENCE

Sawchuk, R.J.; Cartier, L.L. Liquid-chromatographic determination of cyclosporin A in blood and plasma. *Clin.Chem.*, **1981**, *27*, 1368-1371

SAMPLE

Matrix: blood, tissue

Sample preparation: Whole blood. 1 mL Whole blood + 300 ng cyclosporine C + 3 mL MeCN:water 50:50 saturated with zinc sulfate, vortex, centrifuge at 6000 rpm for 10 min, wash the supernatant twice with 2 mL portions of hexane, add the supernatant to a 1 mL 100 mg Bond Elut LRC C18 SPE cartridge, wash with 3 mL portions of MeCN:water 35:65, elute with 1 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in 350 μ L MeCN:water 50:50, inject a 150 μ L aliquot. Tissue. Homogenize 50-100 mg liver or spleen with 300 ng cyclosporine C in MeCN:water 35:65 containing 5% zinc sulfate, centrifuge, wash the supernatant twice with 2 mL portions of hexane, add the supernatant to a 1 mL 100 mg Bond Elut LRC C18 SPE cartridge, wash with 3 mL portions of MeCN:water 35:65, elute with 1 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in 350 μ L MeCN:water 50:50, inject a 150 μ L aliquot.

HPLC VARIABLES

Guard column: Adsorbosphere Direct-Connect guard column

Column: 250 \times 4.6 5 μ m Ultrasphere RP-18

Mobile phase: Gradient. MeCN:water 50:50 for 20 min, to 65:35 over 5 min, maintain at 65:35 over 15 min, to 70:30 over 10 min, maintain at 70:30 for 7 min, wash column with 85:15 for 15 min, re-equilibrate for 10 min.

Column temperature: 70

Injection volume: 150

Detector: UV 214

CHROMATOGRAM

Internal standard: cyclosporine C

Limit of detection: 15 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; whole blood; SPE; pharmacokinetics; liver; spleen

REFERENCE

Wacher, V.J.; Liu, T.; Roberts, J.P.; Ascher, N.L.; Benet, L.Z. Time course of cyclosporine and its metabolites in blood, liver and spleen of naive Lewis rats: Comparison with preliminary data obtained in transplanted animals. *Biopharm. Drug Dispos.*, **1995**, *16*, 303–312

SAMPLE

Matrix: formulations

Sample preparation: 50 mg Paste + 10 mL acetone, mix by inverting at 30 rpm for 15 min, centrifuge at 2500 rpm for 10 min. Remove 4 mL of the upper layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μ L ethyl acetate, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:40 mM pH 5 KH_2PO_4 , 60:10:30

Column temperature: 70

Flow rate: 2

Injection volume: 5

Detector: UV 214

CHROMATOGRAM

Retention time: 8.1

KEY WORDS

gel; paste; stability-indicating

REFERENCE

Ghnassia, L.T.; Yau, D.F.; Kaye, K.I.; Duggin, G.G. Stability of cyclosporine in an extemporaneously compounded paste. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2204–2207

SAMPLE

Matrix: microsomal incubations

Sample preparation: 500 μ L Microsomal incubation + 500 μ L MeCN, mix, centrifuge, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: ultra sepharose ODS (Beckman)

Column: 250 \times 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: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 3 mL MeCN:MeOH:5% (?) zinc sulfate 20:30:50, add benzo[a]pyrene 9,10-diol, mix, centrifuge at 600 g. Add the supernatant to a Sep-Pak C18 SPE cartridge, wash with 2 mL water, elute with 4 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere octyl

Mobile phase: Gradient. MeCN:THF:pH 3 phosphoric acid 30:5:65 for 5 min, to 35:5:60 over 23 min, to 57:5:38 over 12 min.

Flow rate: 1

Detector: UV 230

CHROMATOGRAM

Internal standard: benzo[a]pyrene 9,10-diol

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; human; liver; SPE

REFERENCE

Gelboin, H.V.; Krausz, K.W.; Goldfarb, I.; Buters, J.T.M.; Yang, S.K.; Gonzalez, F.J.; Korzekwa, K.R.; Shou, M. Inhibitory and non-inhibitory monoclonal antibodies to human cytochrome P450 3A3/4. *Biochem.Pharmacol.*, **1995**, *50*, 1841-1850

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac C18

Mobile phase: Gradient. MeCN:water from 30:70 to 70:30 over 40 min, maintain at 70:30 for 10 min, to 90:10 over 2.5 min, to 70:30 over 7.5 min.

Column temperature: 70

Flow rate: 1

Detector: Radioactivity

CHROMATOGRAM

Retention time: 39.2

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

tritium labeled

REFERENCE

Gan, L.-S.L.; Moseley, M.A.; Khosla, B.; Augustijns, P.F.; Bradshaw, T.P.; Hendren, R.W. CYP3A-like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells. Interaction between the two biochemical barriers to intestinal transport. *Drug Metab.Dispos.*, **1996**, *24*, 344-349

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 15 μm Vydac C18 peptide/protein

Mobile phase: Gradient. MeCN:1% acetic acid from 30:70 to 70:30 over 40 min, maintain at 70:30 for 10 min, to 90:10 over 2.5 min, to 70:30 over 7.5 min.

Column temperature: 70

Flow rate: 0.05

Detector: MS, Sciex API-III, IonSpray (pneumatically assisted electrospray)

CHROMATOGRAM

Retention time: 39

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

tritium labeled; microbore

REFERENCE

Gan, L.-S.L.; Moseley, M.A.; Khosla, B.; Augustijns, P.F.; Bradshaw, T.P.; Hendren, R.W. CYP3A-like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells. Interaction between the two biochemical barriers to intestinal transport. *Drug Metab.Dispos.*, **1996**, *24*, 344-349

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: solutions

HPLC VARIABLES

Column: 150 mm long 5 μ m Ultrasphere octyl

Mobile phase: MeCN:water 67:33

Flow rate: 1

Detector: UV 250

CHROMATOGRAM

Retention time: 7.5

REFERENCE

Choi, H.-K.; Flynn, G.L.; Amidon, G.L. Percutaneous absorption and dermal delivery of cyclosporin A. *J.Pharm.Sci.*, **1995**, *84*, 581–583

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Pham-Huy, C.; Sadeg, N.; Becue, T.; Martin, C.; Mahuzier, G.; Warnet, J.M.; Hamon, M.; Claude, J.R. In vitro metabolism of cyclosporin A with rabbit renal or hepatic microsomes: analysis by HPLC-FPIA and HPLC-MS. *Arch.Toxicol.*, **1995**, *69*, 346–349

Mangold, J.B.; Schran, H.F.; Tse, F.L.S. Pharmacokinetics and metabolism of cyclosporin G in humans. *Drug Metab.Dispos.*, **1994**, *22*, 873–879 [cyclosporine G; plasma; urine; feces; column temp 70; gradient]

Nishikawa, T.; Hasumi, H.; Susuki, S.; Kubo, H.; Ohtani, H. Interconversion of cyclosporin molecular form inducing peak broadening, tailing and splitting during reversed-phase liquid chromatography. *Chromatographia*, **1994**, *38*, 359–364 [cyclosporin A; column temp 0-60]

Poirier, J.-M.; Lebot, M.; Cheymol, G. Cyclosporine in whole blood: Drug monitoring difficulties and presentation of a reliable normal-phase liquid chromatographic assay. *Ther.Drug Monit.*, **1994**, *16*, 388–394 [whole blood; normal-phase; column temp 60; SPE]

Maguire, S.; Kyne, F. A rapid selective high-performance liquid chromatography assay for cyclosporine. *Ann.Clin.Biochem.*, **1993**, *30*, 488–489

Sukhanov, A.V.; Shoikhet, I.N. [The determination of cyclosporin A concentration in whole blood by high-pressure liquid chromatography on the Milikhrom-1 microcolumn chromatograph]. *Klin.Lab.Diagn.*, **1993**, *7*–9

Annesley, T.M.; Matz, K.; Leichtman, A.B. High-performance liquid chromatographic analysis of cyclosporin G (Nva2-cyclosporine) in human blood. *Ther.Drug Monit.*, **1992**, *14*, 397–401

Raghuveeran, C.D.; Gopalan, N.; Dangi, R.S.; Kaushik, M.P.; Venkateswaran, K.S. Preparative scale high-performance liquid chromatography and identification of cyclosporine-A from an indigenous fungal isolate. *J.Liq.Chromatogr.*, **1992**, *15*, 2407–2416 [cyclosporine A; fungus; fermentation broth; preparative; gradient; column temp 75]

Svinarov, D.A.; Dimova, M.N. Liquid chromatographic determination of cyclosporine-A in blood, with Chromosorb P columns used for sample purification. *J.Liq.Chromatogr.*, **1991**, *14*, 1683–1690 [cyclosporine A; whole blood; cyclosporine D (IS); column temp 60]

Bowers, L.D. Cyclosporine analysis by high-performance liquid chromatography: precision, accuracy, and minimum detectable quantity. *Transplant.Proc.*, **1990**, *22*, 1150–1154

Gupta, S.K.; Benet, L.Z. HPLC measurement of cyclosporine in blood plasma and urine and simultaneous measurement of its four metabolites in blood. *J.Liq.Chromatogr.*, **1989**, *12*, 1451–1462 [cyclosporine A; plasma; urine; simultaneous metabolites; LOD 30 ng/mL; cyclosporine D (IS); column temp 70]

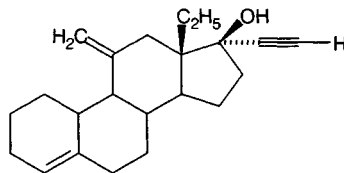
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- Moyer, T.P.; Charlson, J.R.; Ebnet, L.E. Improved chromatography of cyclosporine. *Ther Drug Monit.*, **1986**, *8*, 466–468 [SPE; LOD 25 ng/mL; whole blood; column temp 60; cyclosporine A; cyclosporine D (IS)]
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Desogestrel

Molecular formula: C₂₂H₃₀O

Molecular weight: 310.5

CAS Registry No.: 54024-22-5



SAMPLE

Matrix: mucosal fluid

Sample preparation: Extract 1 mL mucosal fluid twice with 5 mL diethyl ether, evaporate to dryness, reconstitute in 100 μ L MeOH, inject an aliquot. (Aqueous layer can be incubated with β -glucuronidase/sulfatase at 37° for 3 h before extraction.)

HPLC VARIABLES

Guard column: present but not specified

Column: 100 \times 8 μ Bondapak C18

Mobile phase: Gradient. MeCN:water from 55:45 to 100:0 over 15 min, maintain at 100:0 for 30 min

Detector: UV 214

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

also for microsomal incubations (*J. Steroid Biochem.* 1990; 35; 281)

REFERENCE

Madden, S.; Back, D.J.; Martin, C.A.; Orme, M.L. Metabolism of the contraceptive steroid desogestrel by the intestinal mucosa. *Br.J.Clin.Pharmacol.*, **1989**, 27, 295–299

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 200 \times 4.5 μ m Nucleosil RP-8

Mobile phase: MeCN:MeOH:water 3:76:21

Flow rate: 1

Injection volume: 20

Detector: UV 205

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: metabolites, ethinyl estradiol, 3-hydroxydesogestrel, 3-ketodesogestrel, 6-ketoethinyl estradiol

REFERENCE

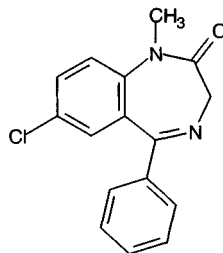
Smilde, A.K.; Bruins, C.H.P.; Doornbos, D.A.; Vink, J. Optimization of the reversed-phase high-performance liquid chromatographic separation of synthetic estrogenic and progestogenic steroids using the multi-criteria decision making method. *J.Chromatogr.*, **1987**, 410, 1–12

Diazepam

Molecular formula: C₁₆H₁₃ClN₂O

Molecular weight: 284.8

CAS Registry No.: 439-14-5



SAMPLE

Matrix: blood

Sample preparation: 1-2 mL Plasma + 200 ng lorazepam + 1 mL 1 M pH 10 bicarbonate buffer + 8 mL n-hexane:ethyl acetate 70:30, shake for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Ultrasphere ODS

Mobile phase: Gradient. A was MeCN:buffer 30:70. B was MeCN:buffer 70:30. A:B from 85:15 to 40:60 over 10 min (Waters curve no. 5), to 0:100 over 5 min (Waters curve no. 1), return to initial conditions over 10 min (Waters curve no. 1). (Buffer was 10 mM pH 3.35 NaH₂PO₄.)

Flow rate: 1

Detector: UV 229

CHROMATOGRAM

Internal standard: lorazepam

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, desmethyldiazepam

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Caraco, Y.; Tateishi, T.; Wood, A.J.J. Interethnic difference in omeprazole's inhibition of diazepam metabolism. *Clin.Pharmacol.Ther.*, **1995**, *58*, 62-72

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 2 μ g/mL prazepam in MeOH + 1 mL saturated trisodium phosphate + 3 mL dichloromethane, shake, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 250 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m CAPCELL PAK C18 (Shiseido)

Mobile phase: MeOH:water 65:35, adjusted to pH 3.4 with phosphoric acid

Column temperature: 30

Flow rate: 0.7

Injection volume: 50

Detector: UV 240

CHROMATOGRAM**Retention time:** 11.8**Internal standard:** prazepam (20.4)**Limit of detection:** 2 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites, demethyldiazepam

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Ishizaki, T.; Chiba, K.; Manabe, K.; Koyama, E.; Hayashi, M.; Yasuda, S.; Horai, Y.; Tomono, Y.; Yamato, C.; Toyoki, T. Comparison of the interaction potential of a new proton pump inhibitor, E3810, versus omeprazole with diazepam in extensive and poor metabolizers of *S*-mephenytoin 4'-hydroxylation. *Clin.Pharmacol.Ther.*, 1995, 58, 155-164

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)) KH₂PO₄ 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:** 6.01**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, acetaminophen, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzo-line, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazoxide, diclofenac, dihydralazine, diphenhydramine, dipyrilamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenpropfen, fentiazac, fencainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, flvoxamine, glibenclamide, glipizide, glutethimide, histapyrrrodine, hydroxychloroquine,

hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nifedipine, niflumic acid, nimodipine, nitrazepam, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ranitidine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vandesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: aceprometazine, aconitine, alprenolol, bisoprolol, diazepam, diltiazem, glibornuride, haloperidol, mianserine, nicardipine, nitrendipine, ramipril, reserpine, tetracaine

KEY WORDS

whole blood; plasma

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: Make 200 μL serum alkaline with borate buffer, extract with cyclohexane:dichloromethane 60:40. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot

HPLC VARIABLES

Column: C18 DB (Supelco)

Mobile phase: MeCN:pH 2.5 phosphate buffer 37:63

Detector: UV 254

OTHER SUBSTANCES

Extracted: bromazepam, clobazam, fluvoxamine, lorazepam, oxazepam

KEY WORDS

serum

REFERENCE

Vandenbergh, H.; MacDonald, J.C. Analysis of fluvoxamine, clobazam and other benzodiazepines on the same HPLC system (Abstract 40). *Ther. Drug Monit.*, **1995**, *17*, 393

SAMPLE

Matrix: blood

Sample preparation: Wash PCPure SPE cartridge (Moritex) containing 0.4 g hydroxyapatite with 10 mL MeCN and remove MeCN by evaporation. 110 μL Plasma + 10 μL of 100 $\mu\text{g}/\text{mL}$ IS in 5% aqueous MeCN, inject onto PCPure cartridge, elute with MeCN: water 1:1. Use first 600 μL of eluate, inject a 20 μL aliquot.

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:** 20**Detector:** UV 240

CHROMATOGRAM**Retention time:** 8**Internal standard:** 4,4'-difluorobenzophenone (15)**Limit of detection:** 0.25 ng

KEY WORDSplasma; 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: 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:** 8.3**Internal standard:** protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, dextromethorphan, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, meprobamate, methadone, methaqualone, mexiletine, norchlorimipramine, nordoxepin, norfluoxetine, nordiazepam, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoyllecgonine, 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, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocanidine, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: chlorimipramine, midazolam

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: Inject 100-200 μ L 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 \times 4 12 μ m TSK-gel G 3 PW (Tosohass); B 75 \times 4.6 Ultrasphere ODS C18 3 μ m

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: 26

Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, estazolam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam

Noninterfering: carbamazepine, ethosuximide, phenobarbital, phenytoin, 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: 1 mL Whole blood or plasma + 1 mL water + 50 μ L 10 μ g/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 μ L initial mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: Gradient. A was 12.5 mM 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: 19.5

Internal standard: papaverine hydrochloride (10.7)

Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, chloroquine, monodesethylchloroquine, nordiazepam

Simultaneous: epinephrine, mefloquine, pyrimethamine, quinine, sulfadoxine

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: Filter (0.5 μ m) serum, inject 200 μ L directly onto column A with mobile phase A, run with mobile phase A for 1.5 min then change to mobile phase B over 0.1 min, wash column A with mobile phase B for 10.5 min, backflush column A onto column B with mobile phase C for 7.5 min then switch column B out of circuit, elute column B with mobile phase C and monitor the eluant, re-equilibrate column A with mobile phase A for at least 5 min.

HPLC VARIABLES

Column: A 15 \times 3.2 5 μ m Brownlee ODS; B 250 \times 1 5 μ m Adsorbosphere ODS

Mobile phase: A 10 mM sodium dodecyl sulfate; B water; C MeOH:water 65:35

Flow rate: A 1; B 1; C 0.06

Injection volume: 200

Detector: UV 242

CHROMATOGRAM

Retention time: 30

Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: nordiazepam, oxazepam, temazepam

KEY WORDS

serum; column-switching; microbore

REFERENCE

Koenigbauer, M.J.; Curtis, M.A. Use of micellar mobile phases and microbore column switching for the assay of drugs in physiological fluids. *J.Chromatogr.*, **1988**, *427*, 277-285

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Plasma + 100 μ L 1 μ g/mL diazepam in MeOH + 2 mL phosphate buffer, vortex for 30 s, add 8 mL hexane:isoamyl alcohol 95:5, shake gently by hand for 10 min, vortex for 1 min, centrifuge at 400 g at 4° for 10 min. Remove organic layer and add it to 2 mL 6 M HCl, shake for 10 min, vortex for 1 min, centrifuge at 400 g for 10 min. Remove the aqueous layer and slowly add about 2 mL 6 M NaOH to it to achieve a pH greater than 7.0, add 2 mL phosphate buffer, vortex for 10 s, add 8 mL hexane:isoamyl alcohol 95:5, shake for 10 min, vortex for 1 min, centrifuge at 400 g for 10 min. Remove the organic layer and evaporate it to dryness at 40° with nitrogen, rinse residue from sides with hexane:isoamyl alcohol 95:5, again dry with nitrogen, take up residue in 40 μ L MeOH, inject a 20-40 μ L aliquot. (Phosphate buffer was 136.1 g KH_2PO_4 in 1 L water, adjusted to pH 7 with 1 M K_2HPO_4 .)

HPLC VARIABLES**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeOH:MeCN:10 mM sodium acetate 40:12.5:47.5, at pH 4.6**Flow rate:** 2.2**Injection volume:** 20-40**Detector:** UV 254**CHROMATOGRAM****Retention time:** 9.2**Internal standard:** diazepam**OTHER SUBSTANCES****Extracted:** lorazepam**Simultaneous:** clonazepam, flunitrazepam, flurazepam, midazolam, nitrazepam, oxazepam, temazepam**KEY WORDS**

plasma; diazepam is IS

REFERENCE

Egan, J.M.; Abernethy, D.R. Lorazepam analysis using liquid chromatography: improved sensitivity for single-dose pharmacokinetic studies. *J.Chromatogr.*, **1986**, *380*, 196-201

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Serum + 2 mL water + 50 μ L 3.2 μ g/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 μ L mobile phase, vortex for 30 s, inject a 50 μ L aliquot.

HPLC VARIABLES**Column:** 50 \times 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: 8.5
Internal standard: estazolam (4)
Limit of detection: 8 ng/mL

OTHER SUBSTANCES

Extracted: clorazepate, nordiazepam, oxazepam, temazepam, triazolam
Simultaneous: bromazepam, flunitrazepam, nitrazepam, sulpride
Noninterfering: haloperidol, trihexyphenidyl

KEY WORDS

serum; pharmacokinetics

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: 2 mL Plasma + 100 μ L 1 μ g/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μ L 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 μ L 250 mM HCl, vortex for 30 s, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 \times 4.6 40 μ m C8 (Supelco)

Column: 250 \times 4.6 5 μ m 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' 5.20

Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES

Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, doxepin, fluphenazine, haloperidol, nortriptyline, oxazepam, thiothixene

Noninterfering: molindone, perphenazine, trifluoperazine

Interfering: imipramine

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:** 200 μL Serum + 200 μL 50 $\mu\text{g}/\text{mL}$ 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:** μ Bondapak 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:** 32.5**Internal standard:** hexobarbital (20.6)**Limit of detection:** 200-2000 ng/mL

OTHER SUBSTANCES**Extracted:** acetaminophen, amobarbital, butobarbital, butalbital, chlordiazepoxide, 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 WORDSserum

REFERENCEKabra, 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, 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 \times 2.1 40 μm preparative grade C18 (Analytichem); B 250 \times 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:** 15.21**Internal standard:** heptanophenone (19.2)

OTHER SUBSTANCES

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

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, CSF, gastric fluid, urine

Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m 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: 14.95

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazeopoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, 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 μ L 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 μ L 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μ L 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 \times 4 30 μ m LiChrocart Aluspher RP-select B (Merck)

Column: 125 \times 4 5 μ m 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; UV 254

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, 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, methyl dopa, 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, tripeleminamine, 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, gastric contents, tissue, urine

Sample preparation: Tissue homogenates were 1:2 in water. 1 mL Sample + 1 mL saturated sodium borate buffer + 100 μ L 20 μ g/mL methyl clonazepam in water + 5 mL n-butyl chloride, rotate at 40 rpm for 30 min, centrifuge at 2500 rcf for 5 min. Remove the organic phase and evaporate it to dryness at 70° under a stream of air, reconstitute the residue in 300 μ L mobile phase, vortex for 30 s, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 2.1 Analytichem ODS with an integral guard column

Mobile phase: MeCN: 100 mM KH_2PO_4 300:700, adjust pH to 3.00 with concentrated phosphoric acid

Column temperature: 60

Flow rate: 1.5

Injection volume: 20

Detector: UV 242

CHROMATOGRAM

Retention time: 7.46

Internal standard: methyl clonazepam (5.36)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: temazepam, trazodone

Also analyzed: acetaminophen, alprazolam, amitriptyline, amoxapine, carbamazepine, chlordiazepoxide, chlorpromazine, chlorprothixene, clonazepam, demoxepam, desipramine, diphenhydramine, disopyramide, doxepin, ethotoin, flurazepam, glutethimide, haloperidol, haloperidol, imipramine, lidocaine, lorazepam, loxapine, maprotiline, mesantoin, mesoridazine, methaqualone, methotrimeprazine, nordiazepam, nortriptyline, oxazepam, pentazocine, perphenazine, phenacetin, phenobarbital, phenytoin, promazine, promethazine, propranolol, protriptyline, salicylic acid, thiothixene, trifluoperazine, trifluoromazine, trimipramine

Noninterfering: chloral hydrate, codeine, ketamine, meperidine, methadone, methamphetamine, methypylron, thioridazine

KEY WORDS

serum; plasma; whole blood

REFERENCE

Root, I.; Ohlson, G.B. Trazodone overdose: report of two cases. *J.Anal.Toxicol.*, **1984**, 8, 91-94

SAMPLE

Matrix: blood, milk

Sample preparation: 500 μ L Plasma or milk + 25 μ L 5 μ g/mL flurazepam in water: MeCN 2.5:97.5 + 500 μ L 67 mM pH 7.4 phosphate buffer + 7 mL diethyl ether, extract for 15 min (A). Remove ether layer and add it to 1 mL 1.5 M HCl, shake for 15 min. Freeze and discard ether phase. Basify aqueous phase with 1 mL 2 M NaOH, extract with 7 mL diethyl ether for 15 min. Evaporate ether at 37° under a stream of nitrogen and take up residue in mobile phase, inject an aliquot. (For plasma **only** ether at (A) can be evaporated at 37° under a stream of nitrogen, take up residue in mobile phase, inject an aliquot.)

HPLC VARIABLES

Guard column: 25 \times 4 5 μ m LiChrospher 60 RP-select B

Column: 125 \times 4 5 μ m LiChrospher 60 RP-select B

Mobile phase: MeCN: 10 mM KH₂PO₄ 31:69, adjusted to pH 2.80 with phosphoric acid

Column temperature: 45

Flow rate: 2

Injection volume: 50

Detector: UV 241

CHROMATOGRAM

Retention time: 8.6

Internal standard: flurazepam (3.0)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: nordiazepam, oxazepam, temazepam

KEY WORDS

plasma; human; rabbit

REFERENCE

Stebler, T.; Guentert, T.W. Determination of diazepam and nordazepam in milk and plasma in the presence of oxazepam and temazepam. *J.Chromatogr.*, **1991**, *564*, 330-337

SAMPLE

Matrix: blood, stomach contents, tissue, urine

Sample preparation: Whole blood, stomach contents. 1 mL Whole blood or stomach contents + IS + 500 μ L 1 M potassium carbonate + 8 mL n-hexane:ethyl acetate 70:30, vortex for 1 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 100 μ L initial mobile phase, vortex, centrifuge, inject a 50 μ L aliquot. Tissue. Cut 1 g tissue into small pieces, make up to 5 mL with water, homogenize (Ultraturrax). 1 mL Homogenate + IS + 500 μ L 1 M potassium carbonate + 8 mL n-hexane:ethyl acetate 70:30, vortex for 1 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 100 μ L initial mobile phase, vortex, centrifuge, inject a 50 μ L aliquot. Urine. 1 mL Urine + IS + 250 μ L concentrated HCl, heat at 100° for 1 h, cool, adjust pH to 9 with NaOH pellets and 1 M potassium carbonate. Add 8 mL n-hexane:ethyl acetate 70:30, vortex for 1 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 100 μ L initial mobile phase, vortex, centrifuge, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 2.1 pellicular reverse phase (Chrompack)

Column: 100 \times 3.5 μ m Chromspher C8 (Chrompack)

Mobile phase: Gradient. MeOH containing 0.03% isopropylamine:water containing 0.03% isopropylamine 20:80 for 2 min, to 30:70 over 0.2 min, maintain at 30:70 for 1.8 min, to 40:60 over 0.2 min, maintain at 40:60 for 0.3 min, to 43:57 over 0.5 min, to 45:55 over 1 min, to 52:48 over 1 min, to 58:42 over 2.5 min, to 75:25 over 1 min, maintain at 75:25 for 4.5 min, return to initial conditions over 0.3 min, re-equilibrate for 3.7 min before next injection.

Flow rate: 0.7

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 10

Internal standard: camazepam (10.5), clotiazepam (11)

Limit of detection: 10 ng/mL

Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Extracted: bromazepam, flunitrazepam, nitrazepam, nordiazepam, oxazepam

Simultaneous: alprazolam, brotizolam, chlordiazepoxide, clonazepam, cloxazolam, flurazepam, loprazolam, lormetazepam, medazepam, prazepam, triazolam

KEY WORDS

whole blood; liver; kidney

REFERENCE

Lambert, W.E.; Meyer, E.; Xue-Ping, Y.; De Leenheer, A.P. Screening, identification, and quantitation of benzodiazepines in postmortem samples by HPLC with photodiode array detection. *J.Anal.Toxicol.*, **1995**, *19*, 35-40

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Serum or urine + 50 ng diazepam-d₅ + 50 ng N-desmethyldiazepam-d₅, add to the SPE cartridge, wash with 2 mL water, elute with 500 µL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 µL mobile phase, inject a 5 µL aliquot. Alternatively, condition a 6 mL narc-2 Bakerbond SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 KH₂PO₄. 1 mL Serum or urine + 50 ng diazepam-d₅ + 50 ng N-desmethyldiazepam-d₅, add to the SPE cartridge at 1 mL/min, wash with 1 mL MeOH:100 mM pH 6.0 KH₂PO₄ 20:80, wash with 1 mL 1 M acetic acid, dry under vacuum for 5 min, wash with 1 mL hexane, dry under vacuum for 1 min, elute with two 2 mL portions of dichloromethane: ammonium hydroxide 96:4 (pH 11). Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 µL mobile phase, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 100 × 2.5 µm LiChrospher 60-RP select B

Mobile phase: MeCN:MeOH:water 1:1:1, pH 6

Flow rate: 0.1

Injection volume: 5

Detector: MS, Finnigan MAT TSQ 7000 triple stage quadrupole, selected reaction monitoring mode (m/z 285/257, offset -30 eV), collision gas argon at 0.27 Pa, heating capillary 250°, repeller 20 V, electrospray capillary 6 kV, electron multiplier 2.3 kV, sheath gas nitrogen at 344.7 kPa, auxiliary gas nitrogen at 5 L/min, octapole offset 5 eV

CHROMATOGRAM

Retention time: 6.4

Internal standard: diazepam-d₅, N-desmethyldiazepam-d₅

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: flunitrazepam, medazepam, nitrazepam

KEY WORDS

SPE; serum

REFERENCE

Kleinschnitz, M.; Herderich, M.; Schreier, P. Determination of 1,4-benzodiazepines by high-performance liquid chromatography-electrospray tandem mass spectrometry. *J.Chromatogr.B*, **1996**, 676, 61–67

SAMPLE

Matrix: blood, urine

Sample preparation: Condition 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: 1.3

Injection volume: 20

Detector: UV 229

CHROMATOGRAM

Internal standard: prazepam

Limit of detection: 63 ng/mL

OTHER SUBSTANCES

Also analyzed: adinazolam, brotizolam, midazolam, nordazepam, oxazepam, temazepam

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: diffusate, tissue

Sample preparation: Homogenize (Polytron PCU-2) 150-200 mg skin with 4 mL chloroform, repeat homogenization, filter (phase-separating paper) extracts. Make the residue alkaline with 2 mL 10% NaOH, extract twice with 4 mL portions of chloroform, wash the extracts twice with 2 mL portions of water, filter (phase-separating paper) the organic layer. Combine all the chloroform layers and evaporate them to dryness under a stream of air, reconstitute the residue in 1 mL mobile phase, filter (microfilter), inject an aliquot.

HPLC VARIABLES

Guard column: 20 × 4 40 μm ODS (Valco)

Column: 150 × 4.6 5 μm Spherisorb ODS-I

Mobile phase: MeCN:water 52:48 containing 10 mM octanesulfonic acid and 1% acetic acid, pH 3.5

Flow rate: 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 6.0

Internal standard: diazepam

OTHER SUBSTANCES

Extracted: physostigmine, tacrine

KEY WORDS

skin; diazepam is IS

REFERENCE

Lau, S.W.J.; Chow, D.; Feldman, S. Simultaneous determination of physostigmine and tetrahydroaminoacridine in a transdermal permeation study by high-performance liquid chromatography. *J.Chromatogr.*, **1990**, *526*, 87–95

SAMPLE

Matrix: formulations

Sample preparation: Stir weighed tablet with 5 mL water until coating is dissolved; add 5 mL 1 mg/mL IS in MeCN; make up to 50 mL with MeCN. Centrifuge an aliquot at 2472 g for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm spherical octyl derivative silica (Merck)

Column: 250 × 4 5 μm spherical octyl derivative silica (Merck)

Mobile phase: MeOH:buffer 70:30 adjusted to pH 6.0 with glacial acetic acid (Buffer was 500 mM sodium acetate and 5 mM sodium 1-heptanesulfonate.)

Column temperature: 50

Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 4.8
Internal standard: n-butyl p-hydroxybenzoate (UV 254)

OTHER SUBSTANCES

Simultaneous: otilonium bromide (UV 290)

KEY WORDS

tablets; stability-indicating

REFERENCE

Mannucci, C.; Bertini, J.; Cocchini, A.; Perico, A.; Salvagnini, F.; Triolo, A. High-performance liquid chromatographic method for assay of otilonium bromide, diazepam, and related compounds in finished pharmaceutical forms. *J.Pharm.Sci.*, **1993**, 82, 367–370

SAMPLE

Matrix: formulations
Sample preparation: Dilute with saline, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Lichrosorb 10 RP 8
Mobile phase: MeOH:THF:water 50:5:50
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: lorazepam, thiopental

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: formulations
Sample preparation: Sonicate 2 mg tablet in MeOH, make up to 20 mL with MeOH, filter (0.45 μ m), dilute 5 mL filtrate to 100 mL with MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 LiChrosorb 10 RP-18
Mobile phase: MeOH:water 60:40
Flow rate: 3.5
Injection volume: 20
Detector: UV 258

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES**Simultaneous:** degradation products, temazepam

KEY WORDStablets

REFERENCE

Gordon, S.M.; Freeston, L.K.; Collins, A.J. Determination of temazepam and its major degradation products in soft gelatin capsules by isocratic reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *368*, 180–183

SAMPLE**Matrix:** hair

Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL MeOH, heat at 55° for 18 h, 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 NaH₂PO₄ + 30 mL diethylamine.)**Flow rate:** 2**Injection volume:** 30**Detector:** UV 214

CHROMATOGRAM**Internal standard:** protriptyline (4)

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, dothiepin, flunitrazepam, haloperidol, imipramine, imipramine, nitrazepam, nortriptyline, oxazepam, temazepam

KEY WORDSmay 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: 1 mL Microsomal incubation + 5 mL dichloromethane, extract, evaporate to dryness, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 Zorbax SB-C18**Mobile phase:** MeCN:MeOH:water 10:40:50**Flow rate:** 1**Detector:** UV 232

CHROMATOGRAM

Internal standard: 6 β -hydroxyprogesterone

OTHER SUBSTANCES

Extracted: metabolites, temazepam

KEY WORDS

rat; human; liver

REFERENCE

Gelboin, H.V.; Krausz, K.W.; Goldfarb, I.; Buters, J.T.M.; Yang, S.K.; Gonzalez, F.J.; Korzekwa, K.R.; Shou, M. Inhibitory and non-inhibitory monoclonal antibodies to human cytochrome P450 3A3/4. *Biochem.Pharmacol.*, **1995**, *50*, 1841–1850

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 20 μ L 10 M NaOH + 100 μ L 70 μ M prazepam in MeOH + 1 mL 100 mM pH 10 sodium carbonate buffer + 5 mL ethyl acetate, rotate for 25 min, centrifuge at 900 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 5 Spherisorb S5 ODS2

Mobile phase: MeOH:water 65:35 containing 0.02% triethylamine, adjusted to pH 7.0 with phosphoric acid

Flow rate: 1

Detector: UV 236

CHROMATOGRAM

Internal standard: prazepam

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver

REFERENCE

Zomorodi, K.; Carlile, D.J.; Houston, J.B. Kinetics of diazepam metabolism in rat hepatic microsomes and hepatocytes and their use in predicting *in vivo* hepatic clearance. *Xenobiotica*, **1995**, *25*, 907–916

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m 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

SAMPLE

Matrix: solutions

Sample preparation: Inject a 30 μ L aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 250 \times 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: 9.5

OTHER SUBSTANCES

Simultaneous: clonazepam, dothiepin, dothiepin sulfoxide, nordiazepam, nordothiepin, nordothiepin sulfoxide

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 \times 4.1 6 μ m 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 32:68

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES

Simultaneous: aspirin, diphenhydramine, o-hydroxyhippuric acid, MPPH, niacin, toluene

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 \times 3.9 4 μ m 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: 16

OTHER SUBSTANCES

Simultaneous: bromazepam, chlordiazepoxide, clobazam, clorazepate, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

REFERENCE

Guillaume, Y.; Guinchart, 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: 12.2

OTHER SUBSTANCES

Simultaneous: bromazepam, chlordiazepoxide, clobazam, clorazepate, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

REFERENCE

Guillaume, Y.; Guinchart, 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: 7.71 (A), 8.83 (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, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flvoxate, 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, methyl-dopa, 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, phenolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozone, 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, sulfapyrazone, 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

some 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 Na₂HPO₄ 50:50

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.32

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, 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

HPLC VARIABLES

Column: 100 × 4.6 3 μm 208HS3410 (Vydac)

Mobile phase: Gradient. MeCN:water from 15:85 to 60:40 over 10 min.

Flow rate: 1.5

Detector: UV 210 (?)

CHROMATOGRAM

Retention time: 9.4

OTHER SUBSTANCES

Simultaneous: barbital, carbamazepine, ethotoin, mephentyoin, methsuximide, phenacemide, phenobarbital, phensuximide

REFERENCE

Vydac HPLC Catalog, **1994-5**, p. 26

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: chlordiazepoxide, desalkylflurazepam, flurazepam, norchlordiazepoxide, nordiazepam, oxazepam, 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 Ci-94, **1994-5**, p. 7.24

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:** 16

OTHER SUBSTANCES**Simultaneous:** bromazepam, chlorazepate, chlordiazepoxide, clobazam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

KEY WORDSconditions are optimized

REFERENCEGuillaume, 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, bromazepam, 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, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropofen, 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, isocarboxystiril, isocarboxazid, isoniazid, isoproter-

enol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephen-
 termine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, metho-
 carbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mi-
 bolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, nor-
 epinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, ox-
 yphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pento-
 barbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, praze-
 pam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic
 acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albu-
 terol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sul-
 facetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, tema-
 zepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theoph-
 ylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic
 acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine,
 triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, tri-
 methoprim, tripelennamine, triproilidine, 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

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 KH₂PO₄ and 4.8 g 85% phos-
 phoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 227; UV 279

CHROMATOGRAM

Retention time: 11.5

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clona-
 zepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, metho-
 carbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone,
 propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zol-
 pidem, 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:** urine**Sample preparation:** 1 mL Urine + 100 μ L 5 mM pH 5.5 acetate buffer + 25 μ L β -glucuronidase/arylsulfatase (0.235/0.065 U, Calbiochem), mix, heat at 37° for 16 h, add 50 μ L 5-50 μ g/mL prazepam in MeOH, add 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove a 2 mL aliquot of the organic layer and add it to 2 mL hexane and 2 mL 6 M HCl, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove 1 mL of the aqueous phase and adjust pH to 6 with 1 mL 6 M NaOH and 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150 μ L mobile phase, inject a 60 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.5 μ m LiChrospher 100 RP-18(e)**Mobile phase:** MeOH:water:triethylamine 30:70:0.1 adjusted to pH 5.5 with phosphoric acid**Flow rate:** 0.7**Injection volume:** 60**Detector:** UV 240

CHROMATOGRAM**Retention time:** 10.3**Internal standard:** prazepam (17.0)**Limit of detection:** 2 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites, desmethyldiazepam, oxazepam, temazepam**Simultaneous:** amitriptyline, caffeine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, flunitrazepam, flurazepam, haloperidol, imipramine, levomepromazine, maprotiline, mianserin, nitrazepam, nortriptyline, perphenazine, phenobarbital, phenytoin, sulpride, thioridazine, triazolam

REFERENCE

Chiba, K.; Horii, H.; Chiba, T.; Kato, Y.; Hirano, T.; Ishizaki, T. Development and preliminary application of high-performance liquid chromatographic assay of urinary metabolites of diazepam in humans. *J. Chromatogr. B*, **1995**, *668*, 77-84

SAMPLE**Matrix:** urine**Sample preparation:** 500 μ L Urine + N-ethylnordiazepam + chlorpheniramine + 100 μ L buffer, centrifuge at 11000 g for 30 s, inject a 500 μ L 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 μ L mobile phase B, with 200 μ L 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 \times 2.1 12-20 μ m PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 \times 3.2 11 μ m Aminex A-28 (Bio-Rad); C 25 \times 3.2 5 μ m C8 (Phenomenex) + 150 \times 4.6 5 μ m 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: 40 (B, C only)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210; UV 235

CHROMATOGRAM

Retention time: k' 1.6

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, amphetamine, benzoylecgonine, caffeine, codeine, cotinine, desipramine, diphenhydramine, ephedrine, flurazepam, hydrocodone, hydromorphone, imipramine, lidocaine, methadone, methamphetamine, morphine, nortriptyline, oxazepam, pentazocine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, secobarbital

Interfering: nordiazepam

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

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Goldnik, A.; Gajewska, M.; Jaworska, M. Determination of oxazepam and diazepam in body fluids by HPLC. *Acta Pol.Pharm.*, **1993**, *50*, 421–422

Kamali, F. Determination of plasma diazepam and desmethyldiazepam by solid-phase extraction and reversed-phase high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1993**, *11*, 625–627

Fernández, P.; Hermida, I.; Bermejo, A.M.; López-Rivadulla, M.; Cruz, A.; Concheiro, L. Simultaneous determination of diazepam and its metabolites in plasma by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1991**, *14*, 2587–2599 [also nordiazepam, oxazepam, temazepam; carbamazepine (IS); SPE]

Gil, M.S.; Ochoa, C.; Vega, S. High performance liquid chromatography of new potential anxiolytic drugs and related benzodiazepines: A comparative study of hydrophobicity. *J.Liq.Chromatogr.*, **1991**, *14*, 2141–2156 [also buspirone, chlordiazepoxide]

Hays, P.A.; Lurie, I.S. Quantitative analysis of adulterants in illicit heroin samples via reversed phase HPLC. *J.Liq.Chromatogr.*, **1991**, *14*, 3513–3517 [simultaneous acetaminophen, acetylcodeine, acetylmorphine, aspirin, benzocaine, caffeine, chloroquine, diamorphine, diphenhydramine, dipyrone, lidocaine, methaqualone, monoacetylmorphine, morphine, nicotinamide, noscapine, papaverine, phenacetin, phenobarbital, phenolphthalein, N-phenyl-2-naphthylamine, salicylic acid, strychnine]

Moro, M.E.; Novillo-Fertrell, J.; Velazquez, M.M.; Rodriguez, L.J. Kinetics of the acid hydrolysis of diazepam, bromazepam, and flunitrazepam in aqueous and micellar systems. *J.Pharm.Sci.*, **1991**, *80*, 459–468

- Brazeau, G.A.; Fung, H.L. Effect of organic cosolvent-induced skeletal muscle damage on the bioavailability of intramuscular [14 C]diazepam. *J.Pharm.Sci.*, **1990**, *79*, 773–777 [rabbit; plasma]
- Noggle, F.T., Jr.; Clark, C.R.; DeRuiter, J. Liquid chromatographic separation of some common benzodiazepines and their metabolites. *J.Liq.Chromatogr.*, **1990**, *13*, 4005–4021 [also metabolites, alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, demoxepam, estazolam, fludiazepam, flunitrazepam, flurazepam, halazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam, triazolam]
- Bastos, M.L.A. Improvement of HPLC conditions for separation of diazepam and its metabolites in biological extracts. *J.Liq.Chromatogr.*, **1989**, *12*, 1919–1934 [also metabolites, desmethyldiazepam, oxazepam, temazepam; prazepam (IS); liver; brain; bile; kidney; gradient]
- Abdel-Hamid, M.E.; Abuirjeie, M.A. Determination of diazepam and oxazepam using high-performance liquid chromatography and fourth-derivative spectrophotometric techniques. *Analyst*, **1988**, *113*, 1443–1446 [powders; tablets; simultaneous degradation products, oxazepam]
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- Wildmann, J. Increase of natural benzodiazepines in wheat and potato during germination. *Biochem.Biophys.Res.Comm.*, **1988**, *157*, 1436–1443 [plants]
- Klockowski, P.M.; Levy, G. Simultaneous determination of diazepam and its active metabolites in rat serum, brain and cerebrospinal fluid by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *422*, 334–339
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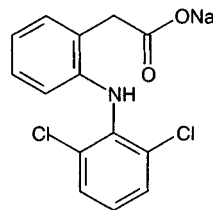
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- Harbin, D.N.; Lott, P.F. The identification of drugs of abuse in urine using reverse phase high pressure liquid chromatography. *J.Liq.Chromatogr.*, **1980**, *3*, 243–256 [urine; also amobarbital, amphetamine, caffeine, chlordiazepoxide, codeine, glutethimide, indole, meperidine, methamphetamine, methaqualone, morphine, pentobarbital, phenobarbital, secobarbital]
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- Wittwer, J.D., Jr. Application of high pressure liquid chromatography to the forensic analysis of several benzodiazepines. *J.Liq.Chromatogr.*, **1980**, *3*, 1713–1724 [simultaneous chlordiazepoxide, clonazepam, clorazepate, cyprazepam, demoxepam, desmethyldiazepam, flurazepam, medazepam, nitrazepam, oxazepam, prazepam; normal phase]

Diclofenac

Molecular formula: C₁₄H₁₁Cl₂NO₂

Molecular weight: 295.1

CAS Registry No.: 15307-79-6 (diclofenac sodium)



SAMPLE

Matrix: aqueous humor

Sample preparation: 100 μ L Aqueous humor + 500 μ L MeCN + 30 μ L 400 ng/mL (+)-naproxen in MeOH, mix mechanically for 90 s, centrifuge at 3000 g for 20 min. Remove the supernatant and dry it under nitrogen at room temperature, dissolve the residue in 50 μ L mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μ L, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m Ultrasphere octyl

Mobile phase: MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, 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: 0.3 ng

OTHER SUBSTANCES

Extracted: flurbiprofen, indomethacin, meclofenamic acid

Simultaneous: bacitracin, cortisone acetate, diazepam, fluorometholone, hydrocortisone acetate, imipramine, ketoprofen, ketorolac tromethamine, levobunolol, metipranolol, neomycin, prednisolone acetate, proparacaine, 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

human; rabbit

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: 500 μ L Plasma + 1 μ g naproxen + 500 μ L 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 μ L MeCN, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 5 5 μ m 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

Sample preparation: 1 mL Plasma + 50 μ L 10 μ g/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 μ L mobile phase, vortex for 30 s, centrifuge at 11500 rpm for 2 min, inject a 140 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-8

Mobile phase: MeCN:water 50:50 adjusted to pH 3.3 with glacial acetic acid (After 13 min increase flow rate to 2.7 mL/min over 4 min, maintain at 2.7 mL/min for 11 min, return to initial conditions.)

Flow rate: 2

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 μ L Plasma + 600 μ L 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 μ L mobile phase, vortex for 30 s, inject a 25 μ L 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

REFERENCELi, 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 NaH₂PO₄ 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 NaH₂PO₄, 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

REFERENCEMason, 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)) KH₂PO₄ 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

OTHER SUBSTANCES

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

Interfering: amitriptyline, diclofenac, mefloquine, nortriptyline, tiocloamarol, trimipramine

KEY WORDS

whole blood; plasma

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: 0.5 mL Plasma + 10 μ L 50 μ g/mL mefenamic acid + 50 μ L 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 μ L mobile phase for 10 s, inject 50 μ L aliquot.

HPLC VARIABLES

Guard column: 30-40 μ m C18 pellicular

Column: 150 \times 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 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 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 bio-availability 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 + 200 μ L 1.5 μ g/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 μ L mobile phase, inject 100 μ L 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 \times 4.6 10 μ m Nucleosil C18; B 150 \times 4.6 10 μ m 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: 50 μ L Plasma + 50 μ L MeCN, mix, centrifuge at 12000 g for 2 min, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 \times 3.2 5 μ m NewGuard ODS

Column: 150 \times 4 5 μ m Nucleosil C18

Mobile phase: MeCN:buffer 32:68 (Buffer was 40 mL 1 M NaH₂PO₄ and 40 mL 500 mM Na₂HPO₄ 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 \times 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; 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, CSF

Sample preparation: Condition a Baker SPE-Octadecyl (C18) SPE cartridge 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) piroprofen, shake, add 1 mL of 1 M phosphoric acid, add to the cartridge, wash twice with 1 mL 1 M phosphoric acid, wash twice with 1 mL water, elute with two 250 μ L aliquots of MeOH. Evaporate MeOH at room temperature under a stream of nitrogen, dissolve residue in 100 μ L mobile phase, inject 10-20 μ L.

HPLC VARIABLES

Column: 80 \times 4.6 3 μ m 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: piroprofen (12)

Limit of detection: 0.7 ng/mL

OTHER SUBSTANCES

Extracted: 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 μ L Plasma or exudate + 50 μ L 50 μ g/mL IS + 250 μ L 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 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 200 \times 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 μ L 24 μ g/mL flurbiprofen + 200 μ L 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 μ L MeOH + 100 μ L water, vortex mix, inject aliquot.

HPLC VARIABLES

Guard column: 20 \times 2 Perisorb RP18 30-40 μ m pellicular

Column: 125 \times 4.6 5 μ m 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.; Patrick, 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 μ L Plasma + 50 μ L 20 μ M mefenamic acid + 200 μ L 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 μ L plasma mobile phase, inject a 100 μ L aliquot. Urine. 100 μ L Urine + 100 μ L 400 μ g/mL ascorbic acid + 100 μ L 5 M NaOH, vortex for 30 s, heat at 75 $^{\circ}$ for 1 h, add 50 μ L 20 μ M mefenamic acid, add 500 μ L 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 μ L urine mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 4 μ m Lichrocart C18 (Merck)

Column: 50 \times 4 4 μ m 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 (\pm)-fluvastatin. *Clin.Pharmacol.Ther.*, **1995**, 58, 412–417

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 \times 4.6 Chromatography Sciences Co. octadecylsilane bonded phase

Mobile phase: MeCN:THF:buffer 300:75:625 (Buffer was 50 mM (NH₄)H₂PO₄ 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.; Lefrançois, 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 μ L Perfusate + 500 μ L MeCN, vortex for 1 min, centrifuge for 10 min, inject a 150 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 100 mm long 5 μ m 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: 125 × 3 Ecocart LiChrospher 100 RP-18

Mobile phase: Isopropanol:100 mM KH₂PO₄: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

OTHER SUBSTANCES

Simultaneous: 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: 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, fenopropfen, 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, mazedol, mefepamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, pimozone, 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, sulfinyprazone, 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

some 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 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, 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, 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, fenpropfen, 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, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, meggestol, mepacrine, meperidine, mephen-termine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, norriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphebutazone, 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, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triproilidine, 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: 250 μL Urine + 50 μL 12.5 $\mu\text{g}/\text{mL}$ 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. After 12 min increase flow rate to 1.6 mL/min over 2 min, maintain at 1.6 mL/min for 12 min, return to initial conditions. (Buffer was 1.156 g $(\text{NH}_4)\text{H}_2\text{PO}_4$ in 1 L water adjusted to pH 2.66 with concentrated phosphoric acid.)**Flow rate:** 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 WORDSpharmacokinetics

REFERENCESawchuk, 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

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- Reer, O.; Bock, T.K.; Müller, B.W. In vitro corneal permeability of diclofenac sodium in formulations containing cyclodextrins compared to the commercial product Voltaren ophtha. *J.Pharm.Sci.*, **1994**, *83*, 1345–1349 [perfusate]
- Zhang, S.Y.; Zou, H.Q.; Zhang, Z.Y.; Peng, W.L.; Liu, L.Q. [High-performance liquid chromatographic method for the determination of diclofenac in serum and its pharmacokinetics in healthy volunteers]. *Yao Hsueh Hsueh Pao*, **1994**, *29*, 228–231
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- De Bernardi di Valserra, M.; Feletti, F.; Tripodi, A.S.; Contos, S.; Carabelli, A.; Maggi, L.; Germogli, R. Pharmacokinetic studies in healthy volunteers on a new gastroprotective pharmaceutical form of diclofenac. *Arzneimittelforschung*, **1993**, *43*, 373–377 [plasma; column temp 30; LOD 20 ng/mL; pharmacokinetics]
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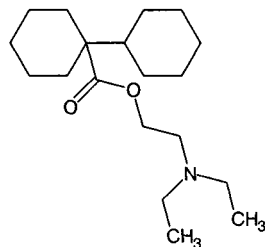
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- Moncrieff, J. Extractionless determination of diclofenac sodium in serum using reversed-phase high-performance liquid chromatography with fluorimetric detection. *J.Chromatogr.*, **1992**, *577*, 185–189 [fluorescence detection; harmol (IS); serum; column temp 40; LOD 20 ng/mL]
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- El-Sayed, Y.M.; Abdel-Hameed, M.E.; Suleiman, M.S.; Najib, N.M. A rapid and sensitive high-performance liquid chromatographic method for the determination of diclofenac sodium in serum and its use in pharmacokinetic studies. *J.Pharm.Pharmacol.*, **1988**, *40*, 727–729
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Dicyclomine

Molecular formula: C₁₉H₃₅NO₂

Molecular weight: 309.5

CAS Registry No.: 77-19-0 (dicyclomine), 67-92-5 (dicyclomine hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μ L 1 μ g/mL dicyclomine in water + 1 mL MeCN, vortex, allow to stand for 10 min, add 200 μ L 1 M pH 9.4 tris(hydroxymethyl)methylamine (TRIS), add 5 mL hexane, shake horizontally for 10 min, centrifuge at 2000 g for 5 min. Remove the aqueous layer and add it to 3 mL hexane, shake horizontally for 10 min, centrifuge at 2000 g for 5 min. Combine the hexane layers and add them to 1 mL 100 mM HCl, shake for 10 min, centrifuge. Remove the aqueous layer and evaporate it to dryness under vacuum, reconstitute in 250 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 100 mm long 5 μ m Techsil CN

Mobile phase: MeOH:20 mM pH 6.2 orthophosphoric acid buffer 40:60

Column temperature: 30

Flow rate: 0.6

Injection volume: 100

Detector: E, ESA Coulochem 5100-A, guard cell 1.0 V, dual porous graphite electrode 0.85 and 0.95 V

CHROMATOGRAM

Retention time: 23

Internal standard: dicyclomine

OTHER SUBSTANCES

Extracted: oxybutynin

KEY WORDS

plasma; dicyclomine is IS

REFERENCE

Hughes, K.M.; Lang, J.C.T.; Lazare, R.; Gordon, D.; Stanton, S.L.; Malone-Lee, J.; Geraint, M. Measurement of oxybutynin and its N-desethyl metabolite in plasma, and its application to pharmacokinetic studies in young, elderly and frail elderly volunteers. *Xenobiotica*, **1992**, *22*, 859–869

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 2 Deltabond C8 (Keystone)

Mobile phase: MeCN:2-butanone:50 mM pH 7.0 phosphate buffer 27:13:60

Flow rate: 0.15

Injection volume: 1

Detector: Chemiluminescence following post-column reaction. A 1 mM solution of Ru(2,2'-bipyridine)₃²⁺ in 50 mM sodium sulfate (continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)₃³⁺ 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 was pumped at 0.3 mL/min and mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

CHROMATOGRAM

Retention time: 14

Limit of detection: 0.1-1 µg/mL

OTHER SUBSTANCES

Simultaneous: cyclobenzaprine

KEY WORDS

post-column reaction

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

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, bamethane, 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, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclizine, 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, tocinide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

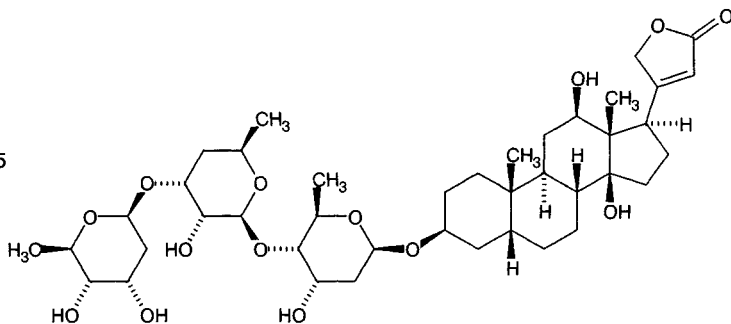
Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection. *J.Chromatogr.*, **1985**, *323*, 191–225

Digoxin

Molecular formula: $C_{41}H_{64}O_{14}$

Molecular weight: 781.0

CAS Registry No.: 20830-75-5



SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Cyclobond I β -cyclodextrin SPE cartridge (Astec) with 2 mL MeOH, 2 mL MeCN, 2 mL isopropanol, and 2 mL water (SPE cartridge A). Condition a 1 mL Cyclobond I β -cyclodextrin SPE cartridge (Astec) with 2 mL MeOH, 2 mL MeCN, and 2 mL dichloromethane (SPE cartridge B). Condition a 1 mL Bond Elut C1 SPE cartridge with 2 mL MeOH and 2 mL MeCN (SPE cartridge C). 1 mL Serum + 10 ng digitoxin + 1 mL water, add to SPE cartridge A, wash with 2 mL water, wash with 1 mL MeOH:7.5 mM pH 7.0 potassium phosphate buffer 20:80, wash with 3 mL water, wash with 1 mL isopropanol:water 10:90, dry under vacuum for 5 min, wash with ten 100 μ L aliquots of dichloromethane, dry under vacuum for 5 min, elute with 1 mL isopropanol. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, add 50 μ L 10% 4-dimethylaminopyridine in MeCN then 50 μ L 4% 1-naphthoyl chloride in MeCN under nitrogen in a glove box (relative humidity <26%), mix thoroughly, heat at 50 $^{\circ}$ for 1 h, centrifuge briefly, evaporate under a stream of nitrogen, add 2 mL 5% pH 10.0 sodium bicarbonate solution, shake for 1 min, add 2 mL chloroform, shake, centrifuge. Remove the organic layer and wash it with 2 mL 5% sodium bicarbonate, wash twice with 2 mL portions of 50 mM HCl, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute with 200 μ L dichloromethane, add to SPE cartridge B, wash with eight 100 μ L aliquots of dichloromethane, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 250 μ L MeCN, add to SPE cartridge C, rinse container with 250 μ L MeCN, add rinse to the SPE cartridge, add 500 μ L MeCN to the SPE cartridge. Collect all the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot. (Purify 4-dimethylaminopyridine by passing a 30% solution in MeCN through a layer of silica gel covered with a layer of activated charcoal, evaporate the filtrate under reduced pressure, store the residue in a desiccator. Immerse glassware in sulfuric acid:nitric acid 80:20 for 24 h, wash with water, treat with 1% Surfasil (Pierce) in toluene, rinse with water, dry in an oven.)

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m silica (Applied Biosystems)

Column: 150 \times 4.6 3 μ m Spherisorb silica

Mobile phase: Hexane:dichloromethane:MeCN:MeOH 36:6.3:5.4:0.2

Flow rate: 1.6

Injection volume: 20

Detector: F ex 217 em 340

CHROMATOGRAM

Retention time: 10

Internal standard: digitoxin (9.5)

Limit of detection: 0.25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, dihydrodigoxin

Noninterfering: acetaminophen, acetazolamide, acyclovir, albuterol, allopurinol, amiodarone, amitriptyline, amoxicillin, ampicillin, aspirin, atenolol, atropine, azathioprine, bumetanide, calcitriol, captopril, carbamazepine, cefazolin, cefoperazone, ceftazidime, ceftiozime, cephalexin, chlorthalidone, ciprofloxacin, clavulanic acid, clindamycin, clonidine, clotrimazole, codeine, conjugated estrogens, cyclophosphamide, diazepam, diphenhydramine, dipyridamole, dobutamine, docusate sodium, dopamine, enalapril, erythromycin, famotidine, fluconazole, furosemide, gemfibrozil, gentamicin, glyburide, heparin, hydralazine, hydrochlorothiazide, ibuprofen, ipratropium bromide, isosorbide dinitrate, isradipine, labetalol, lidocaine, lorazepam, lovastatin, medroxyprogesterone acetate, meperidine, metoclopramide, metolazone, metoprolol, midazolam, minoxidil, morphine, nicotine, nifedipine, nitroglycerin, norepinephrine, nystatin, oxybutynin, oxycodone, pentoxifylline, phenytoin, piroxicam, prednisone, procainamide, procaine, promethazine, propoxyphene, ranitidine, sotalol, spironolactone, sulbactam, sulfamethoxazole, sulfisoxazole, temazepam, tetracycline, timolol, tobramycin, triamcinolone acetonide, triamterene, trimethoprim, vancomycin, verapamil, warfarin

KEY WORDS

normal phase; derivatization; serum; SPE; pharmacokinetics

REFERENCE

Tzou, M.-C.; Sams, R.A.; Reuning, R.H. Specific and sensitive determination of digoxin and metabolites in human serum by high-performance liquid chromatography with cyclodextrin solid-phase extraction and precolumn fluorescence derivatization. *J. Pharm. Biomed. Anal.*, **1995**, *13*, 1531–1540

SAMPLE

Matrix: blood

Sample preparation: 3 mL Serum + 20 μ L 8 μ g/mL IS in EtOH + 3 mL acetone, vortex for 20 s, centrifuge at 1000 g for 5 min, remove the supernatant and add it to 2 mL iso-octane:dichloromethane 80:20, vortex for 1 min, centrifuge at 1000 g for 5 min. Remove the acetone/water layer and evaporate it to 3 mL under a stream of nitrogen at 37°, add 10 mL dichloromethane:n-propanol 98:2, rotate for 10 min, centrifuge at 1000 g for 5 min, repeat extraction, filter the organic layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μ L MeOH:water 50:50, inject the whole amount.

HPLC VARIABLES

Guard column: 15 \times 3.2 ODS (Brownlee)

Column: 150 \times 4.6 3 μ m Spherisorb ODS II

Mobile phase: MeOH:EtOH:isopropanol:buffer 52:3:1:45 (Prepare buffer by mixing 12.5 mL 0.15% hydrogen peroxide in water with 500 mL 500 μ g/mL L-ascorbic acid in water, stir for 2 h. Prepare fresh each week.)

Flow rate: 0.4

Injection volume: 100

Detector: F ex 360 (filter) em 425 (filter) following post-column reaction. The column effluent mixed with concentrated HCl pumped at 0.5 mL/min and flowed through a 20 m \times 0.3 mm i.d. PTFE coil at 79 \pm 1° to the detector. (The flow of concentrated HCl was generated by displacing concentrated HCl from a pressure vessel with hexane. The hexane was pumped into the pressure vessel by an HPLC pump.)

CHROMATOGRAM

Retention time: 18.5

Internal standard: digitoxigenin (25.5)

Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES

Simultaneous: digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, dihydrodigoxigenin, dihydrodigoxin, furosemide, spironolactone

Noninterfering: mexiletine, captopril, dipyridamole, disopyramide, procainamide, propafenone, quinidine, sulfamethoxazole, trimethoprim, verapamil

KEY WORDS

serum; post-column reaction

REFERENCE

Embree, L.; McErlane, K.M. Development of a high-performance liquid chromatographic-post-column fluorogenic assay for digoxin in serum. *J.Chromatogr.*, **1989**, 496, 321-334

SAMPLE

Matrix: blood

Sample preparation: Wash a C18 Sep-Pak with 24 mL MeOH then 24 mL water. Wash a Diol Sep-Pak with 6 mL MeOH. 300 μ L Serum + 25 μ L 23.86 nmol/L deslanoside + 25 μ L 28.68 μ mol/L gitoxigenin, vortex, add 300 μ L to the C18 Sep-Pak, wash with 1 mL water, 1 mL ice-cold 100 g/L ZnSO₄, 1 mL 20 mL/L MeCN, 3 mL water, remove excess water by applying vacuum for several min. Elute the C18 Sep-Pak with 3 mL MeOH through the Diol Sep-Pak and collect the eluate. Dry the eluate under nitrogen at 37°, reconstitute in 200 μ L mobile phase, vortex 30 s, centrifuge at 1100 g for 15 min, inject 185-195 μ L. After each run clean column by injecting 200 μ L MeOH + 1 mL THF. Immunoassay detection. Gitoxigenin elutes at 16 min as a marker. Collect deslanoside (10-12 min), digoxin (24-30 min), and 1 min fractions on either side of digoxin (4 tubes). Dry under air at 25°, reconstitute residue with 230 μ L digoxin-free serum, vortex 20 s, centrifuge at 1100 g for 5 min, use 200 μ L. Determine digoxin by fluorescence polarization immunoassay in accordance with manufacturer's instructions.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m ODS2 (Chromatography Sciences Co.)

Mobile phase: THF:water 20.5:79.5

Flow rate: 0.6

Injection volume: 185-195

Detector: UV 218; Immunoassay

CHROMATOGRAM

Retention time: 25

Internal standard: deslanoside

OTHER SUBSTANCES

Noninterfering: dexamethasone, hydroxyprogesterone, methylprednisolone, progesterone

Interfering: cortisone (with UV assay), fludrocortisone (with UV assay), prednisone (with UV assay)

KEY WORDS

serum

REFERENCE

Stone, J.A.; Soldin, S.J. Improved liquid chromatographic/immunoassay of digoxin in serum. *Clin.Chem.*, **1988**, 34, 2547-2551

SAMPLE

Matrix: blood

Sample preparation: 3 mL Plasma + 3 mL acetone, vortex, centrifuge at 1000 g for 5 min, remove the supernatant and add it to 2 mL isoctane, vortex, centrifuge at 1000 g

for 5 min. Remove the acetone/water layer and evaporate it to 3 mL under a stream of nitrogen at 37°, add 10 mL dichloromethane:n-propanol 98:2, rotate for 10 min, centrifuge at 1000 g for 5 min, filter the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μ L MeOH:water 50:50, inject the whole amount.

HPLC VARIABLES

Guard column: 37 μ m ODS

Column: 150 \times 4.6 3 μ m Spherisorb ODS II

Mobile phase: MeOH:EtOH:isopropanol:water 52:3:1:45

Flow rate: 0.3

Injection volume: 100

Detector: F ex 360 (filter) em 425 (filter) following post-column reaction. The column effluent mixed with the reagent and flowed through a 10 m \times 0.3 mm i.d. knitted PTFE coil at 79 \pm 1° to the detector. The reagent was generated by mixing 1.1 mM hydrogen peroxide in 0.1% ascorbic acid pumped at 0.038 mL/min and concentrated HCl pumped at 0.192 mL/min and allowing this mixture to flow through a 2 m \times 0.8 mm i.d. PTFE coil to the point where it mixed with the column effluent (*J.Chromatogr.* 1986, 377, 233).

CHROMATOGRAM

Retention time: 33

Internal standard: digitoxigenin (42)

Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Simultaneous: digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, dihydrodigoxigenin, dihydrodigoxin, furosemide, spironolactone

Noninterfering: captopril, dipyridamole, disopyramide, procainamide, propafenone, quinidine, sulfamethoxazole, trimethoprim, verapamil

KEY WORDS

plasma; post-column reaction; comparison with RIA

REFERENCE

Kwong, E.; McErlane, K.M. Analysis of digoxin at therapeutic concentrations using high-performance liquid chromatography with post-column derivatization. *J.Chromatogr.*, **1986**, 381, 357-363

SAMPLE

Matrix: blood, perfusate

Sample preparation: 200 μ L Plasma or perfusate + 20 μ L 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 μ L mobile phase, inject a 50 μ L 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 (17 α -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: Urine. Extract 20 mL urine with 20 mL dichloromethane containing 3% heptafluorobutanol for 15 min, centrifuge at 1000 g for 10 min. Remove 15 mL aqueous phase, extract with 15 mL dichloromethane containing 3% heptafluorobutanol for 15 min, centrifuge at 1000 g for 10 min. Combine 10 mL volumes of each organic phase, evaporate under nitrogen to about 0.5 mL, add 20 μ L 1-pentanol, evaporate to 20 μ L, dissolve residue in 250 μ L mobile phase, inject a 100 μ L aliquot. Plasma. 10 mL Plasma + 3 g sodium chloride, extract with 15 mL dichloromethane containing 3% heptafluorobutanol for 15 min, centrifuge at 1000 g for 10 min. Evaporate 10 mL organic phase under nitrogen to about 0.5 mL, add 25 μ L phosphate buffer, evaporate to 25 μ L, dissolve residue in mobile phase, inject whole amount. (Plasma extraction in *Acta Pharmacol. Toxicol.* (Copenh.) 1986, 59 (Suppl. 4), 1-62.)

HPLC VARIABLES

Column: 150 \times 4.5 7 μ m LiChrosorb RP-8

Mobile phase: Isopropanol:pH 6.3 phosphate buffer (I=0.1) 16.5:83.5

Flow rate: 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 23

Limit of detection: 2 ng/mL

KEY WORDS

plasma

REFERENCE

Eriksson, B.-M.; Tekensbergs, L.; Magnusson, J.-O.; Molin, L. Determination of tritiated digoxin and metabolites in urine by liquid chromatography. *J.Chromatogr.*, **1981**, *223*, 401-408

SAMPLE

Matrix: bulk

Sample preparation: Dissolve a small amount in 200 μ L dry pyridine, add 15 mg 3,5-dinitrobenzoyl chloride, shake for 2 h, evaporate to dryness under nitrogen under reduced pressure. Reconstitute with 1.5 mL ethyl acetate, wash 4 times with 1 mL portions of 5% sodium bicarbonate containing 2.5 mg/mL 4-dimethylaminopyridine, wash 4 times with 1 mL portions of 1% HCl, wash 4 times with 1 mL portions of water, evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot (*J.Chromatogr.Sci.* 1983, 21, 495).

HPLC VARIABLES

Guard column: 15 \times 3.2 Brownlee ODS

Column: 150 \times 4.6 3 μ m Spherisorb ODS II

Mobile phase: MeOH:EtOH:MeCN:isopropanol:100 mM pH 4.6 sodium acetate buffer 40:3:60:2:22

Flow rate: 1

Detector: UV 254; E, ESA Coulochem Model 5100A, Model 5020 guard cell -0.8 V (placed before the injector), Model 5010 dual-electrode analytical cell with glassy-carbon electrodes (-0.8 V first electrode, +0.8 V second electrode)

CHROMATOGRAM**Retention time:** 13**Limit of detection:** 0.39 ng (electrochemical detection)

OTHER SUBSTANCES**Simultaneous:** digoxigenin bisdigoxoside, digoxigenin monodigoxoside, digoxigenin, dihydrodigoxigenin, dihydrodigoxin

KEY WORDS

derivatization

REFERENCEEmbree, L.; McErlane, K.M. Electrochemical detection of 3,5-dinitrobenzoyl derivatives of digoxin by high-performance liquid chromatography. *J. Chromatogr.*, **1990**, 526, 439–446

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 not more than 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:** 6**Limit of detection:** 11 ng/mL (100 µL injection)

OTHER SUBSTANCES**Simultaneous:** diginatin, diginatin, digitoxigenin, digitoxin, digoxigenin, gitaloxigenin, gitaloxin, gitoxigenin, gitoxin, lanatoside A, lanatoside B, lanatoside C, lanatoside D, lanatoside E

KEY WORDS

ampoules; normal phase; derivatization

REFERENCE

Nachtmann, F.; Spitz, 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. Place 1 mL 100 ng/mL digitoxin in isopropanol in a tube and evaporate. Add 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 μ L 1-naphthoyl chloride, add 100 μ L 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 μ g/mL clindamycin in water to stop bacterial metabolism, homogenize with mechanical shaking for 15 min. Evaporate 1 mL 100 ng/mL digitoxin in isopropanol into a tube, weigh ca. 1 g homogenate into the tube, add 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 μ L 1-naphthoyl chloride, add 100 μ L 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 \times 4.6 3 μ m 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 (372 nm max)

CHROMATOGRAM

Retention time: 9.4

Internal standard: digitoxin (8.1)

Limit of detection: 5 ng/mL (urine); 50 ng/g (feces)

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

normal phase

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: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Partisil ODS III C18

Mobile phase: MeCN:water:phosphoric acid 35:65:0.1

Flow rate: 2

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Noninterfering: amrinone

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

SAMPLE

Matrix: formulations

Sample preparation: One tablet (0.25 mg digoxin) + 5 mL acetone:ethanol 9:1 containing 0.11826 mg dexamethasone, sonicate 5 min, centrifuge at 1400 g for 5 min, evaporate supernatant under vacuum, dissolve residue in 100 μ L MeOH, inject 0.2 μ L aliquots.

HPLC VARIABLES

Column: 95 \times 0.5 Japan Spectroscopic SC-01 (5 μ m 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: 10

Internal standard: dexamethasone (14)

OTHER SUBSTANCES

Simultaneous: digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, dimethyldigoxin, β -methyldigoxin

KEY WORDS

tablets; microbore

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: 1 mL Sample + 9 mL mobile phase, mix, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Zorbax ODS

Mobile phase: MeCN:phosphoric acid:water 26:0.1:58, adjusted to pH 6.5 with 10 M NaOH

Flow rate: 2
Injection volume: 20
Detector: UV 218

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: digoxigenin, digoxigenin didigitoxoside, digoxigenin monodigitoxoside
Noninterfering: milrinone

KEY WORDS

stability-indicating; 5% dextrose; injections

REFERENCE

Riley, C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection. *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091

SAMPLE

Matrix: formulations

Sample preparation: Directly inject a 20 μL aliquot of a 250 $\mu\text{g}/\text{mL}$ digoxin injection.

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeCN:water 29:71

Flow rate: 2

Injection volume: 20

Detector: UV 218

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: mercaptobenzothiazole

KEY WORDS

injections

REFERENCE

Reepmeyer, J.C.; Juhl, Y.H. Contamination of injectable solutions with 2-mercaptobenzothiazole leached from rubber closures. *J.Pharm.Sci.*, **1983**, *72*, 1302–1305

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10 μL aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 250 \times 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 μ L reaction coil (Dionex) to the detector.

CHROMATOGRAM

Retention time: 9

Limit of detection: 230 ng/mL

OTHER SUBSTANCES

Simultaneous: digitoxigenin, digitoxigenin bisdigitoxoside, digitoxigenin monodigitoxoside, digitoxin, digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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, bromazepam, 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, debrisquinone, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, 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, isocarboxtyril, 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, mephen-

termine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, nor-epinephrine, 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, pyrihydione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, 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

Guard column: 37 μm ODS

Column: 150 \times 4.6 3 μm Spherisorb ODS II

Mobile phase: MeOH:EtOH:isopropanol:water 52:3:1:45

Flow rate: 0.3

Detector: F ex 360 (filter) em 425 (filter) following post-column reaction. The column effluent mixed with the reagent and flowed through a 10 m \times 0.3 mm i.d. knitted PTFE coil at $79 \pm 1^\circ$ to the detector. The reagent was generated by mixing 1.1 mM hydrogen peroxide in 0.1% ascorbic acid pumped at 0.038 mL/min and concentrated HCl pumped at 0.192 mL/min and allowing this mixture to flow through a 2 m \times 0.8 mm i.d. PTFE coil to the point where it mixed with the column effluent.

CHROMATOGRAM

Retention time: 33

OTHER SUBSTANCES

Simultaneous: digitoxigenin, digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, dihydrodigoxigenin, dihydrodigoxin, furosemide, spironolactone

KEY WORDS

post-column reaction

REFERENCE

Kwong, E.; McErlane, K.M. Development of a high-performance liquid chromatographic assay for digoxin using post-column fluorogenic derivatization. *J. Chromatogr.*, 1986, 377, 233-242

SAMPLE**Matrix:** urine**Sample preparation:** 10 mL Urine + 2 mL 1 M HCl (check pH is 1-2), heat 37° for 3 h, add 5 mL pH 6.5 phosphate buffer, add 2 mL 1 M NaOH (check pH is 6.5-7.0). Add to a 20 cm Extrelut SPE column, rinse flask with 3 mL water, add rinsings to column, dry for 15 min, elute with 40 mL dichloromethane, evaporate eluent to dryness, dry over concentrated sulfuric acid. Prepare a 100 mg/mL solution of 4-nitrobenzoyl chloride (4-NBP) in dry pyridine with gentle heating. Use immediately. Dissolve residue from column in 30 μ L dry pyridine, add 20 μ L 2 mg/mL digitoxigenin in pyridine, add 300 μ L 4-NBP solution, shake well. Heat at 70° for 1 h, add 2 mL 5% sodium bicarbonate, shake until precipitate has dissolved, add 2 mL chloroform, shake, centrifuge, repeat extraction twice. Combine chloroform layers, wash three times with 2 mL 1 M HCl, inject an aliquot of chloroform solution directly.

HPLC VARIABLES**Column:** 200 \times 4 Hibar 5 μ m Lichrosorb Si 60**Mobile phase:** n-Hexane:dichloromethane:methanol 82.9:14.2:2.9**Flow rate:** 1.2**Injection volume:** 20**Detector:** UV 258

CHROMATOGRAM**Retention time:** 12**Internal standard:** digitoxigenin (8)**Limit of detection:** 1 μ g/mL

KEY WORDS

normal phase; derivatization; SPE; digoxin is hydrolysed to digoxigenin and determined as its 4-NBP derivative

REFERENCEJakobsen, P.; Waldorff, S. Determination of digoxin, digoxigenin and dihydrodigoxigenin in urine by extraction, derivatization and high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *382*, 349-354

SAMPLE**Matrix:** urine**Sample preparation:** 10 mL Urine + 0.5 mL 20 μ g/mL digitoxigenin in dichloromethane + 20 mL dichloromethane, shake for 15 min, centrifuge for 20 min. Remove the organic phase and add it to 15 mL 5% sodium bicarbonate, shake for 15 min, centrifuge for 20 min. Remove the organic phase and evaporate it to dryness at 50° under a stream of nitrogen, add 200 μ L derivatizing solution to the residue, shake gently at room temperature for 10 min, evaporate to dryness under a stream of nitrogen at 50°, add 2 mL 2 mg/mL 4-dimethylaminopyridine in 5% sodium bicarbonate, shake for 5 min, add 1 mL chloroform, rock on an Aliquot Mixer. Remove the organic phase and add it to 2 mL 5% sodium bicarbonate solution, mix for 2 min. Remove the organic phase and add it to 3 mL 50 mM HCl containing 5% NaCl, mix for 2 min. Remove the organic phase and repeat the acid wash 3 more times, inject a 100 μ L aliquot of the organic phase. (The derivatizing solution was 85 mg/mL 3,5-dinitrobenzoyl chloride in pyridine, prepared with gentle warming to help the solid dissolve.)

HPLC VARIABLES**Column:** 250 \times 4.6 10 μ m Partisil 10**Mobile phase:** Hexane:dichloromethane:MeCN 60:20:20**Flow rate:** 1.8

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 54

Internal standard: digitoxigenin (17)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: digoxigenin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside

KEY WORDS

normal phase

REFERENCE

Bockbrader, H.N.; Reuning, R.H. Digoxin and metabolites in urine: A derivatization-high-performance liquid chromatographic method capable of quantitating individual epimers of dihydrodigoxin. *J.Chromatogr.*, **1984**, *310*, 85–95

SAMPLE

Matrix: urine

Sample preparation: Extract 20 mL urine with 20 mL dichloromethane containing 3% heptafluorobutanol for 15 min, centrifuge at 1000 g for 10 min. Remove 15 mL aqueous phase, extract with 15 mL dichloromethane containing 3% heptafluorobutanol for 15 min, centrifuge at 1000 g for 10 min. Combine 10 mL volumes of each organic phase, evaporate under nitrogen to about 0.5 mL, add 20 μ L 1-pentanol, evaporate to 20 μ L, dissolve residue in 250 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m LiChrosorb SI 60

Mobile phase: n-Heptane:1-pentanol:MeCN:water 64:26:9:1

Flow rate: 1.5

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 15

Limit of detection: 10 ng/mL

KEY WORDS

normal phase

REFERENCE

Eriksson, B.-M.; Tekensbergs, L.; Magnusson, J.-O.; Molin, L. Determination of tritiated digoxin and metabolites in urine by liquid chromatography. *J.Chromatogr.*, **1981**, *223*, 401–408

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Ikeda, Y.; Fujii, Y.; Nakaya, I.; Yamazaki, M. Quantitative HPLC analysis of cardiac glycosides in *Digitalis purpurea* leaves. *J.Nat.Prod.*, **1995**, *58*, 897–901

Hui, J.; Geraets, D.R.; Chandrasekaran, A.; Wang, Y.-M.C.; Caldwell, J.H.; Robertson, L.W.; Donnerberg, R.L.; Reuning, R.H. Digoxin disposition in elderly humans with hypochlorhydria. *J.Clin.Pharmacol.*, **1994**, *34*, 734–741 [urine; feces; derivatization; fluorescence detection; normal phase; digitoxin (IS); extracted metabolites; LOD 5 ng/mL]

Oosterkamp, A.J.; Irth, H.; Beth, M.; Unger, K.K.; Tjaden, U.R.; van de Greef, J. Bioanalysis of digoxin and its metabolites using direct serum injection combined with liquid chromatography and on-line

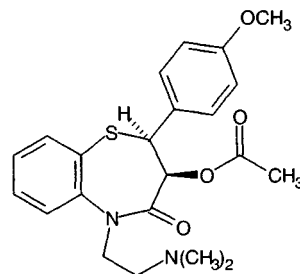
- immunochemical detection. *J.Chromatogr.B*, **1994**, *653*, 55–61 [LOD 160 pg/mL; serum; column-switching; post-column reaction; fluorescence detection; extracted metabolites; pharmacokinetics]
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- Nakashima, H.; Tsutsumi, K.; Hashiguchi, M.; Kumagai, Y.; Ebihara, A. Determination of β -methyl digoxin and its metabolites by high-performance liquid chromatography and fluorescence polarization immunoassay. *J.Chromatogr.*, **1989**, *489*, 425–431
- Fujii, Y.; Ikeda, Y.; Yamazaki, M. Micro high-performance liquid chromatographic determination of cardiac glycosides in β -methyl digoxin and digoxin tablets. *J.Chromatogr.*, **1988**, *448*, 157–164
- Reh, E. Determination of digoxin in serum by on-line immunoabsorptive clean-up high-performance liquid chromatographic separation and fluorescence-reaction detection. *J.Chromatogr.*, **1988**, *433*, 119–130 [serum; column-switching; LOD 300 pg/mL; post-column reaction; fluorescence detection]
- Desta, B. Separation of digoxin from dihydrodigoxin and the other metabolites by high-performance liquid chromatography with post-column derivatization. *J.Chromatogr.*, **1987**, *421*, 381–386 [post-column reaction; fluorescence detection; simultaneous metabolites]
- Plum, J.; Daldrup, T. Detection of digoxin, digitoxin, their cardioactive metabolites and derivatives by high-performance liquid chromatography and high-performance liquid chromatography-radioimmunoassay. *J.Chromatogr.*, **1986**, *377*, 221–231 [gradient; tissue; extracted metabolites; SPE]
- Vetticaden, S.J.; Barr, W.H.; Beightol, L.A. Improved method for assaying digoxin in serum using high-performance liquid chromatography-radioimmunoassay. *J.Chromatogr.*, **1986**, *383*, 187–193 [serum; dog; RIA detection]
- de Jong, H.C.; Voogt, W.H.; Bos, P.; Frei, R.W. Tensammetric detection in high performance liquid chromatography. Application to lynestrenol and some cardiac glycosides. *J.Liq.Chromatogr.*, **1983**, *6*, 1745–1758 [also lynestrenol; electrochemical detection]
- Gandelman, M.S.; Birks, J.W. Liquid chromatographic detection of cardiac glycosides, saccharides and hydrocortisone based on the photoreduction of 2-tert-butylantraquinone. *Anal.Chim.Acta*, **1983**, *155*, 159–171 [post-column reaction; simultaneous diginatin; also hydrocortisone; LOD 2 ng]
- Wagner, J.G.; Dick, M.; Behrendt, D.M.; Lockwood, G.F.; Sakmar, E.; Hees, P. Determination of myocardial and serum digoxin concentrations in children by specific and nonspecific assay methods. *Clin.Pharmacol.Ther.*, **1983**, *33*, 577–584 [heart; tissue; serum; ethoxzolamide (IS); RIA detection]
- Desta, B.; Kwong, E.; McErlane, K.M. Separation of digoxin, digitoxin and their potential metabolites, impurities or degradation products by high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *240*, 137–143
- Gault, H.; Kalra, J.; Ahmed, M.; Kepkay, D.; Longerich, L.; Barrowman, J. Influence of gastric pH on digoxin biotransformation. II. Extractable urinary metabolites. *Clin.Pharmacol.Ther.*, **1981**, *29*, 181–190 [urine; radioactivity detection; tritium labeled]
- Loo, J.C.K.; McGilveray, I.J.; Jordan, N. The estimation of serum digoxin by combined HPLC separation and radioimmunological assay. *J.Liq.Chromatogr.*, **1981**, *4*, 879–886 [serum; RIA detection; extracted metabolites]

Diltiazem

Molecular formula: C₂₂H₂₆N₂O₄S

Molecular weight: 414.5

CAS Registry No.: 42399-41-7 (diltiazem), 33286-22-5 (diltiazem hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 200 μ L 500 ng/mL 4-methylpropranolol in solvent + 1 mL buffer, mix briefly, add 5 mL MTBE, shake vigorously for 10 min, centrifuge at 2500 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness using a vortex evaporator at 30°, reconstitute the residue in 2 mL hexane and 200 μ L solvent, vortex for 2 min, discard the upper hexane layer, wash again with 2 mL hexane, inject a 100 μ L aliquot of the aqueous phase. (Buffer was 200 mM K₂HPO₄ adjusted to pH 10 with 5 M KOH. Solvent was MeCN:MeOH:10 mM pH 2 sulfuric acid 10:45:45 containing 56 mM sodium octanesulfonate. MTBE was stored over activated charcoal and filtered (Whatman No. 2v) immediately before use. Hexane was purified by stirring 4 volumes hexane with 1 volume concentrated sulfuric acid overnight then washing twice with 1 volume water.)

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Suplex pKb-100 (Supelco)

Column: 150 \times 4.6 5 μ m Suplex pKb-100 (Supelco)

Mobile phase: MeCN:MeOH:10 mM pH 2 sulfuric acid 10:45:45 containing 10 mM sodium octanesulfonate

Flow rate: 1

Injection volume: 200

Detector: UV 237

CHROMATOGRAM

Retention time: 10.3

Internal standard: 4-methylpropranolol (Wyeth-Ayerst) (17.1)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, quinidine (F ex 247 em 270)

KEY WORDS

plasma

REFERENCE

Carignan, G.; Carrier, K.; Laganière, S.; Lessard, M. Simultaneous determination of diltiazem and quinidine in human plasma by liquid chromatography. *J.Chromatogr.B*, **1995**, 672, 261–269

SAMPLE

Matrix: blood

Sample preparation: Make serum alkaline with 10% sodium carbonate, extract with diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!). Remove the organic layer and extract it with 10 mM HCl, inject an aliquot of the aqueous layer.

HPLC VARIABLES**Column:** 150 × 4.6 5 μm Supelcosil LC-CN**Mobile phase:** MeCN:water:500 mM KH₂PO₄ 36:62:2**Flow rate:** 1.8**Detector:** UV 210

CHROMATOGRAM**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** mexiletine, propafenone

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

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)) KH₂PO₄ 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 238

CHROMATOGRAM**Retention time:** 5.90**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, atenolol, benazepril, benperidol, benzocaine, benzoyllecgonine, bepridil, betaxolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazoxide, diclofenac, dihydralazine, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin,

doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glipizide, glutethimide, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephesisin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nifedipine, niflumic acid, nimodipine, nitrazepam, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ranitidine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: aconitine, astemizole, bisoprolol, diazepam, diltiazem, glibornuride, haloperidol, medifoxamine, mianserine, nicardipine, nitrendipine, ramipril, reserpine, vinblastine

KEY WORDS

whole blood; plasma

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: Wash a Lida 100 mg C18 SPE column with 3 mL MeCN then 3 mL 100 mM ammonium dihydrogen phosphate. 1 mL Plasma + 20 μ L 7.5 μ g/mL propyldiltiazem in MeOH + 500 μ L 100 mM ammonium dihydrogen phosphate, vortex, add to SPE column, wash with 2 mL MeCN:water 20:80, wash with 1 mL MeCN:water 40:60, air dry column for 30 s, elute with 500 μ L MeCN:100 mM ammonium dihydrogen phosphate 80:20 containing 0.06% triethylamine (final pH 6.8). Evaporate eluate to dryness under nitrogen at 40–45°, dissolve residue in 250 μ L MeCN:50 mM KH_2PO_4 pH 2.9 20:80, inject 100 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 40 μ m Pelliguard LC8

Column: 150 \times 4.6 5 μ m Hypersil C8 BDS

Mobile phase: MeCN:50 mM KH_2PO_4 adjusted to pH 2.9 with phosphoric acid:triethylamine 398:600:2

Flow rate: 1

Injection volume: 100

Detector: UV 238

CHROMATOGRAM

Retention time: 6

Internal standard: propyldiltiazem (8)

Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES**Simultaneous:** metabolites

KEY WORDSplasma; SPE

REFERENCE

Ascalone, V.; Locatelli, M.; Malavasi, B. Determination of diltiazem and its main metabolites in human plasma by automated solid-phase extraction and high-performance liquid chromatography: a new method overcoming instability of the compounds and interference problems. *J.Chromatogr.B*, **1994**, *657*, 133–140

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 5 mL MTBE + 40 μ L 10 μ g/mL verapamil in MeOH, vortex, centrifuge at 3000 g for 15 min. Remove the supernatant and add it to 80 μ L 50 mM sulfuric acid, vortex, centrifuge, inject a 25 μ L aliquot of the lower aqueous phase.

HPLC VARIABLES**Guard column:** 42 \times 3 30-35 μ m CO-PELL ODS**Column:** 250 \times 4.6 10 μ m Econosil-CN**Mobile phase:** MeOH:50 mM ammonium dihydrogen phosphate:triethylamine 45:55:0.25, pH adjusted to 5.0 with 1 M phosphoric acid**Injection volume:** 25**Detector:** UV 237

CHROMATOGRAM**Internal standard:** verapamil

KEY WORDSplasma; pharmacokinetics

REFERENCE

Bialer, M.; Hadad, S.; Golomb, G.; Barel, S.; Samara, E.; Abu Salach, O.; Berkman, N.; Danenberg, H.D.; Ben David, J.; Caron, D. Pharmacokinetic analysis of two new sustained-release products of diltiazem designed for twice- and once-daily treatment. *Biopharm.Drug Dispos.*, **1994**, *15*, 45–52

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Serum + 200 μ L 1 M NaOH, vortex 30 s, dilute to 5 mL with MeCN, vortex 2 min, centrifuge at 2200 g for 5 min, inject a 100 μ L aliquot of supernatant.

HPLC VARIABLES**Guard column:** 23 \times 3.6 10 μ m Waters C8**Column:** 300 \times 4.1 10 μ m LiChrosorb RP-8**Mobile phase:** MeCN:10 mM Na₂HPO₄ + 0.1% triethanolamine 40:60, pH adjusted to 3.0 \pm 0.1 with 85% phosphoric acid**Flow rate:** 1.2**Injection volume:** 100**Detector:** UV 237

CHROMATOGRAM**Retention time:** 7

Limit of detection: 2.5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

serum

REFERENCE

Chaudhary, R.S.; Gangwal, S.S.; Avachat, M.K.; Shah, Y.N.; Jindal, K.C. Determination of diltiazem hydrochloride in human serum by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *614*, 261–266

SAMPLE

Matrix: blood

Sample preparation: 0.5 mL Plasma + hexane:2-propanol 98:2, stir 15 min, centrifuge 1500 g 10 min. Remove organic layer and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute residue in 200 μ L mobile phase, vortex 1 min, inject 20 μ L aliquot.

HPLC VARIABLES

Guard column: 18 \times 0.4 5 μ m Ultron ES-OVM

Column: 150 \times 4.6 5 μ m Ultron ES-OVM (ovomucoid chemically bonded to aminopropyl-silica gel)

Mobile phase: EtOH:20 mM KH_2PO_4 3:97, pH adjusted to 4.5 with phosphoric acid or KOH

Flow rate: 1

Injection volume: 20

Detector: UV 237

CHROMATOGRAM

Retention time: 3.5 (cis-(+)-diltiazem), 7.5 (cis-(-)-diltiazem)

Limit of detection: 64 ng/mL

KEY WORDS

plasma; chiral; effect of organic modifier and pH on retention time and separation is discussed

REFERENCE

Rosell, G.; Camacho, A.; Parra, P. Direct enantiomeric separation of cis-(\pm)diltiazem in plasma by high-performance liquid chromatography with ovomucoid column. *J.Chromatogr.*, **1993**, *619*, 87–92

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μ L 3 μ g/mL propranolol hydrochloride in water + 6 mL MTBE, shake 15 min, centrifuge at 1500 g for 15 min. Remove organic layer and add it to 100 μ L 0.05 M sulfuric acid, shake 15 min, centrifuge at 1500 g at 4° for 10 min, discard organic layer, inject 50 μ L aliquots of aqueous layer.

HPLC VARIABLES

Column: 100 \times 2 5 μ m ODS Hypersil

Mobile phase: MeCN:10 mM Na_2HPO_4 40:60 containing 40 mM sodium dodecyl sulfate and 3 mM tetrabutylammonium bromide, adjusted to pH 2 with orthophosphoric acid

Flow rate: 0.5

Injection volume: 50

Detector: UV 240

CHROMATOGRAM**Retention time:** 6**Internal standard:** propranolol (10.5)**Limit of detection:** 1 ng/mL

OTHER SUBSTANCES**Simultaneous:** metabolites

KEY WORDSplasma; microbore

REFERENCE

Zoest, A.R.; Hung, C.T.; Wanwimolruk, S. Diltiazem: a sensitive HPLC assay and application to pharmacokinetic study. *J.Liq.Chromatogr.*, **1992**, *15*, 1277-1287

SAMPLE**Matrix:** blood

Sample preparation: 2 mL Plasma + 100 μ L 6 μ g/mL imipramine in 10 mM HCl + 200 μ L 10% ammonium carbonate (final pH 8.7), vortex gently, add 5 mL MTBE, extract (Vibrax VXR2) for 20 min, centrifuge at 4° at 1720 g for 10 min, remove the organic layer. Add 5 mL dichloromethane to the aqueous layer, shake for 20 min on a reciprocating shaker, centrifuge at 0° at 1720 g for 10 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L 10 mM HCl, wash with 2 mL MTBE, wash with 2 mL hexane, inject a 3-20 μ L aliquot of the aqueous layer.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Ultrasphere-ODS C18**Mobile phase:** MeCN:MeOH:40 mM ammonium acetate 24:40:36 containing 0.04% triethylamine, pH adjusted to 7.3 with glacial acetic acid**Flow rate:** 1.2**Injection volume:** 3-20**Detector:** UV 237

CHROMATOGRAM**Retention time:** 13.0**Internal standard:** imipramine (17.9)

OTHER SUBSTANCES**Extracted:** metabolites**Simultaneous:** alprazolam, amitriptyline, desipramine, loxapine, nortriptyline**Noninterfering:** clomipramine

KEY WORDSplasma; pharmacokinetics

REFERENCE

Yeung, P.K.F.; Montague, T.J.; Tsui, B.; McGregor, C. High-performance liquid chromatographic assay of diltiazem and six of its metabolites in plasma: application to a pharmacokinetic study in healthy volunteers. *J.Pharm.Sci.*, **1989**, *78*, 592-597

SAMPLE**Matrix:** blood

Sample preparation: 500 μ L Serum + 250 μ L di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μ L 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 240

CHROMATOGRAM

Retention time: 6

Internal standard: minaprine (5.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, nortriptyline

Also analyzed: amiodarone, clomipramine, desipramine, haloperidol, imipramine, propafenone, 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, 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:** bulk**Sample preparation:** Inject a 10 μ L aliquot of a 1 mg/mL diltiazem solution containing 1 mg/mL IS.

HPLC VARIABLES**Column:** 250 \times 4.6 10 μ m Chiralcel OF cellulose tris(4-chlorophenylcarbamate)**Mobile phase:** Hexane:isopropanol 50:50 containing 0.1% diethylamine**Column temperature:** 30**Flow rate:** 1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 9 ((-)-trans), 11 ((-)-cis), 15 ((+)-trans), 19 ((+)-cis)**Internal standard:** (+)-cis-5-acetyl-2,3-dihydro-3-hydroxy-2-(p-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (acetylthiazepin) (24)

KEY WORDS

chiral

REFERENCEIshii, K.; Minato, K.; Nakai, H.; Sato, T. Simultaneous assay of four stereoisomers of diltiazem hydrochloride. Application to in vitro chiral inversion studies. *Chromatographia*, **1995**, *41*, 450–454

SAMPLE**Matrix:** formulations**Sample preparation:** Powder tablets, weigh out powder equivalent to about 50 mg diltiazem, dissolve in 100 mL MeOH, filter. Remove 5 mL aliquot, add 5 mL 0.5 mg/mL cyproheptadine hydrochloride in MeOH, make up to 50 mL with water, inject 50 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Rexchome 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:** 6**Internal standard:** cyproheptadine (8)

KEY WORDS

tablets; stability-indicating

REFERENCEShivram, 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:** perfusate

HPLC VARIABLES**Column:** 100 × 8 4 μm Novapak C18**Mobile phase:** MeCN:0.092% phosphoric acid containing 0.2% triethylamine 26:74**Flow rate:** 2**Detector:** UV 214

CHROMATOGRAM**Internal standard:** lidocaine**Limit of quantitation:** 10 ng/mL

OTHER SUBSTANCES**Simultaneous:** metabolites, diphenhydramine**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

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), 5.42 (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, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, mida-

zolam, 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, sulfapyrazone, 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

some 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 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, cortisolone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropofen, 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, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid,

medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methylprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, 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, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triproilidine, 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 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: 9.08

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, 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: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)

Mobile phase: Hexane:isopropanol 90:10

Flow rate: 0.5

Detector: UV

CHROMATOGRAM

Retention time: 21 (-), 28 (+)

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

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Hussain, M.D.; Tam, Y.K.; Gray, M.R.; Coutts, R.T. Mechanisms of time-dependent kinetics of diltiazem in the isolated perfused rat liver. *Drug Metab.Dispos.*, **1994**, *22*, 36–42 [perfusate; extracted metabolites; LOQ 30 nM; bupivacaine (IS)]

Ishii, K.; Minato, K.; Nishimura, N.; Miyamoto, T.; Sato, T. Direct chromatographic resolution of four optical isomers of diltiazem hydrochloride on a Chiralcel OF column. *J.Chromatogr.*, **1994**, *686*, 93–100 [chiral; column temp 10-40]

Rutledge, D.R.; Abadi, A.H.; Lopez, L.M. Liquid chromatographic determination of celiprolol, diltiazem, desmethyldiltiazem and deacetyldiltiazem in plasma using a short alkyl chain silanol deactivated column. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 135–140 [extracted metabolites, celiprolol; LOD 3 ng/mL]

Sigusch, H.; Henschel, L.; Kraul, H.; Merkel, U.; Hoffmann, A. Lack of effect of grapefruit juice on diltiazem bioavailability in normal subjects. *Pharmazie*, **1994**, *49*, 675–679 [pharmacokinetics; SPE; extracted metabolites; flurazepam (IS)]

Rutledge, D.R.; Abadi, A.H.; Lopez, L.M.; Beaudreau, C.A. High-performance liquid chromatographic determination of diltiazem and two of its metabolites in plasma using a short alkyl chain silanol deactivated column. *J.Chromatogr.*, **1993**, *615*, 111–116 [plasma; extracted metabolites; imipramine IS; LOD 4 ng/mL; pharmacokinetics; interfering theophylline; simultaneous desipramine, propranolol, verapamil; non-interfering aspirin, atenolol, caffeine, ibuprofen, lidocaine, metoprolol, nifedipine]

Hubert, P.; Chiap, P.; Crommen, J. Automatic determination of diltiazem and deacetyldiltiazem in human plasma using liquid-solid extraction on disposable cartridges coupled to HPLC—Part I: optimization of the HPLC system and method validation. *J.Pharm.Biomed.Anal.*, **1991**, *9*, 877–882

Hubert, P.; Chiap, P.; Crommen, J. Automatic determination of diltiazem and deacetyldiltiazem in human plasma using liquid-solid extraction on disposable cartridges coupled to HPLC—Part II: optimization of liquid- solid extraction. *J.Pharm.Biomed.Anal.*, **1991**, *9*, 883–887

Ishii, K.; Banno, K.; Miyamoto, T.; Kakimoto, T. Determination of diltiazem hydrochloride enantiomers in dog plasma using chiral stationary-phase liquid chromatography. *J.Chromatogr.*, **1991**, *564*, 338–345 [dog; plasma; chiral; achiral; extracted metabolites; column temp 40]

Jensen, B.H.; Larsen, C. Quantitation of diltiazem in human plasma by HPLC using an end-capped reversed-phase column. *Acta Pharm.Nord.*, **1991**, *3*, 179–180

Leneuve, A.; Stheneur, A.; Bousquet, A.; Roux, A. Automated high-performance liquid chromatographic technique for determining diltiazem and its three main metabolites in serum. *J.Liq.Chromatogr.*, **1991**, *14*, 3519–3530 [serum; extracted metabolites; SPE; pharmacokinetics; LOD 2.5 ng/mL]

Bonnefous, J.L.; Bouliou, R. Comparison of solid-phase extraction and liquid-liquid extraction methods for liquid chromatographic determination of diltiazem and its metabolites in plasma.

- J.Liq.Chromatogr.*, **1990**, *13*, 3799–3807 [extracted metabolites; plasma; SPE; propionyldeacetyldiltiazem (IS); LOD 5 ng/mL]
- Boulieu, R.; Bonnefous, J.L.; Ferry, S. Determination of diltiazem and its metabolites in plasma by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1990**, *13*, 291–301 [plasma; extracted metabolites; propionyldeacetyldiltiazem (IS); LOD 5 ng/mL]
- Boulieu, R.; Bonnefous, J.L.; Ferry, S. Solid-phase extraction of diltiazem and its metabolites from plasma prior to high-performance liquid chromatography. *J.Chromatogr.*, **1990**, *523*, 542–546 [plasma; propionyldeacetyldiltiazem (IS); SPE; extracted metabolites; LOD 0.3 ng; non-interfering diazepam, flunitrazepam, midazolam, nifedipine, pancuronium bromide, procainamide, propranolol, quinidine, verapamil]
- Johnson, K.E.; Pieper, J.A. An HPLC method for the determination of diltiazem and three of its metabolites in serum. *J.Liq.Chromatogr.*, **1990**, *13*, 951–960 [extracted metabolites; serum; doxepin (IS); LOD 3 ng/mL; non-interfering carbamazepine, chlorpromazine, gallopamil, imipramine, lidocaine, prochlorperazine, quinidine, thioridazine, trimeprazine; pharmacokinetics]
- Parissi-Poulou, M.; Ismailos, G.; Macheras, P. Modified HPLC analysis of diltiazem in plasma for pharmacokinetic studies. *Int.J.Pharm.*, **1990**, *62*, R13–R16
- Shah, Y.; Khanna, S.; Dighe, V.S.; Jindal, K.C. High-performance liquid chromatographic determination of diltiazem hydrochloride in tablets. *Indian Drugs*, **1990**, *27*, 363–364
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- Rustum, A.M. Determination of diltiazem in human whole blood and plasma by high-performance liquid chromatography using a polymeric reversed-phase column and utilizing a salting-out extraction procedure. *J.Chromatogr.*, **1989**, *490*, 365–375
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- Ascalone, V.; Dal Bo', L. Automated high-performance liquid chromatographic and column-switching technique for on-line clean-up and analysis of diltiazem in human plasma. *J.Chromatogr.*, **1987**, *423*, 239–249
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- Johnson, S.M.; Wahba Khalil, S.K. An HPLC method for the determination of diltiazem and desacetyldiltiazem in human plasma. *J.Liq.Chromatogr.*, **1987**, *10*, 673–685 [plasma; extracted desacetyldiltiazem (IS); LOD 5 ng/mL]

- iltiazem; diazepam (IS); LOD 2 ng/mL; non-interfering atenolol, chlorthalidone, furosemide, hydralazine, methyldopa, pentoxifylline; also captopril, chlorothiazide, dipyridamole, disopyramide, isosorbide dinitrate, labetalol, metoprolol, pindolol, procainamide, propranolol, quinidine, warfarin]
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- Goebel, K.J.; Kollé, E.U. High-performance liquid chromatographic determination of diltiazem and four of its metabolites in plasma. Application to pharmacokinetics. *J.Chromatogr.*, **1985**, *345*, 355–363
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- Verghese, C.; Smith, M.S.; Aanonsen, L.; Pritchett, E.L.; Shand, D.G. High-performance liquid chromatographic analysis of diltiazem and its metabolite in plasma. *J.Chromatogr.*, **1983**, *272*, 149–155
- Hussain, M.D.; Tam, Y.K.; Finegan, B.A.; Coutts, R.T. Simple and sensitive high-performance liquid chromatographic method for the determination of diltiazem and six of its metabolites in human plasma. *J.Chromatogr.*, **1992**, *582*, 203–209 [plasma; benzylamphetamine (IS); extracted metabolites; pharmacokinetics; LOQ 5 ng/mL; non-interfering bupivacaine, diphenhydramine, lidocaine, metoprolol]

Doxazosin

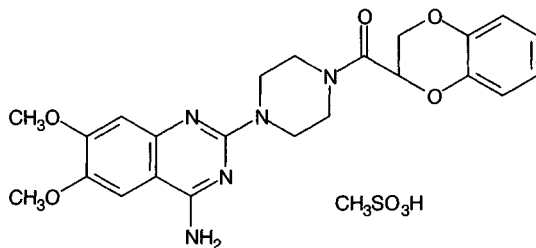
Molecular formula: C₂₃H₂₅N₅O₅

Molecular weight: 451.5

CAS Registry No.: 74191-85-8

(doxazosin),

77883-43-3 (doxazosin mesylate)



SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma + 50 µL 1 µg/mL propranolol in MeOH + 250 µL MeOH, agitate briefly, let stand for 10 min, centrifuge at 3000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL MeOH:water 30:70, wash with 1 mL water, elute with 1 mL MeOH:acetic acid 99.5:0.5. Evaporate the eluate to dryness under a stream of air, reconstitute the residue in 150 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Zorbax CN

Mobile phase: MeOH:buffer 50:50 (Buffer was 10 mM perchloric acid and 1.8 mM sodium heptanesulfonate.)

Flow rate: 1

Detector: F ex 245 em bandpass 320-390 (Corning 7-60 filter)

CHROMATOGRAM

Retention time: 5

Internal standard: propranolol (3.5)

Limit of detection: 1 ng/mL

KEY WORDS

plasma; pharmacokinetics; SPE

REFERENCE

Jackman, G.P.; Colagrande, F.; Louis, W.J. Validation of a solid-phase extraction high-performance liquid chromatographic assay for doxazosin. *J.Chromatogr.*, **1991**, *566*, 234–238

SAMPLE

Matrix: blood

Sample preparation: 250 µL Serum + 250 µL 20 ng/mL prazosin in MeOH:water 30:70, vortex for 30 s, add 1.5 mL ethyl acetate, vortex for 15 s, centrifuge at 3000 rpm for 2 min. Remove the organic layer and evaporate it to dryness in a vortex evaporator at 40° for 10 min, reconstitute the residue in 200 µL MeOH:water 50:50, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 50 × 3.9 40 µm glass beads

Column: 150 × 4.6 alumina-based reversed-phase gamma RP-1 (ES Industries)

Mobile phase: MeCN:MeOH:25 mM pH 10.9 sodium carbonate 15:15:70 (At the end of each day flush with about 75 mL MeOH:water 50:50 until pH of effluent is neutral.)

Flow rate: 2

Injection volume: 50

Detector: F ex 246 em 389 (cutoff filter)

CHROMATOGRAM

Retention time: 4.9

Internal standard: prazosin (1.8)

Limit of quantitation: 0.5 ng/mL

KEY WORDS

serum

REFERENCE

Fouda, H.G.; Twomey, T.M.; Schneider, R.P. Liquid chromatographic analysis of doxazosin in human serum with manual and robotic sample preparation. *J.Chromatogr.Sci.*, **1988**, *26*, 570–573

SAMPLE

Matrix: blood

Sample preparation: 1 mL Whole blood + 5 mL diethyl ether, shake for 10 min, centrifuge at 2000 rpm for 5 min, freeze in acetone/dry ice. Remove the organic layer and add it to 100 μ L 50 mM sulfuric acid, shake for 10 min, centrifuge at 2000 rpm for 5 min, inject a 20 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 \times 4.5 μ m Spherisorb ODS

Mobile phase: MeOH:water 55:45 containing 10 mM pentane sodium sulfate and 9 mM tetramethylammonium chloride, adjusted to pH 3.4 with glacial acetic acid

Flow rate: 1.8

Injection volume: 20

Detector: F ex 254 em 400 (cut-off filter)

CHROMATOGRAM

Retention time: 9

Internal standard: doxazosin

OTHER SUBSTANCES

Extracted: trimazosin

KEY WORDS

whole blood; doxazosin is IS

REFERENCE

Hughes, M.A.; Meredith, P.A.; Elliott, H.L. The determination of trimazosin and its metabolite CP23445 in whole blood by high performance liquid chromatography using fluorescence detection. *J.Pharmacol.Methods*, **1984**, *12*, 29–34

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.6 12 μ m 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' 96.16

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyriline (mepyr-amine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleppamine, 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: 125 × 5 5 μ m Spherisorb C8

Mobile phase: MeCN:water 25:45 containing 5 mM dibutylamine

Flow rate: 2

Detector: F ex 346 em 340 (filter)

REFERENCE

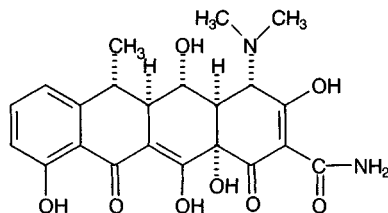
Ferry, D.G.; Caplan, N.B.; Cubeddu, L.X. Interaction between antidepressants and alpha 1-adrenergic receptor antagonists on the binding to alpha 1-acid glycoprotein. *J.Pharm.Sci.*, **1986**, *75*, 146–149

Doxycycline

Molecular formula: C₂₂H₂₄N₂O₈

Molecular weight: 444.4

CAS Registry No.: 564-25-0, 17086-28-1 (monohydrate),
24390-14-5 (HCl monohydrate), 83038-87-3 (fosfatex),
24390-14-5 (hydrate)



SAMPLE

Matrix: blood

Sample preparation: 100 µL Serum + 50 µL 6% aqueous ascorbic acid + 50 ng demeclocycline in MeOH + 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 NaH₂PO₄ and 2 M Na₂SO₃, pH 6.1.)

HPLC VARIABLES

Guard column: 4 µm Nova-Pak C18 Guard-Pak

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: 4.2

Internal standard: demeclocycline (2.7)

Limit of quantitation: 20 ng/mL

KEY WORDS

serum

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

Sample preparation: 100 µL Plasma + 20 µL trifluoroacetic acid, mix 30 s in a whirl mixer, centrifuge at 5400 g for 5 min, inject supernatant (80 µL).

HPLC VARIABLES

Guard column: 10 µm Waters RP phenyl

Column: 125 × 4.6 10 µm Waters RP phenyl

Mobile phase: MeCN:10 mM phosphoric acid 30:70

Flow rate: 2

Injection volume: 80

Detector: UV 270

CHROMATOGRAM

Retention time: 2.2

Limit of detection: 15 ng/mL

KEY WORDS

plasma

REFERENCE

Krämer-Horaczynska, F. High-performance liquid chromatographic procedures for the quantitative analysis of 15 tetracycline derivatives in small blood samples. *J.Chromatogr.Sci.*, **1991**, *29*, 107-113

SAMPLE**Matrix:** blood, urine

Sample preparation: Serum. 0.5 mL Serum + 0.5 mL MeCN:85% phosphoric acid:water 20:2:78, vortex, filter (10 000 or 30 000 Da cutoff) by centrifuging at 2200 g for 30 min, inject 10 μ L aliquot of filtrate. Urine. Dilute urine 5 to 10 times with MeCN:85% phosphoric acid:water 20:1.7:78.3, vortex, filter (10 000 or 30 000 Da cutoff) by centrifuging at 2200 g for 30 min, inject 10 μ L aliquot of filtrate.

HPLC VARIABLES**Column:** 220 \times 4.6 phenyl**Mobile phase:** MeOH:MeCN:triethylamine:phosphoric acid:80 mM pH 2.4 sodium phosphate buffer 10:1.5:0.5:1.7:86.3**Column temperature:** 50**Flow rate:** 0.6-0.8**Injection volume:** 10**Detector:** UV 268; UV 345

CHROMATOGRAM**Retention time:** 8**Limit of detection:** <10 ng/mL

KEY WORDS

serum; cow

REFERENCE

Riond, J.L.; Hedeem, K.M.; Tyczkowska, K.; Riviere, J.E. Determination of doxycycline in bovine tissues and body fluids by high-performance liquid chromatography using photodiode array ultraviolet-visible detection. *J.Pharm.Sci.*, **1989**, *78*, 44-47

SAMPLE**Matrix:** blood, urine

Sample preparation: Serum. Condition a Bond-Elut C18 SPE cartridge with 1 volume MeOH and 2 volumes water. 1 mL Serum + 5 mL buffer, add to SPE cartridge, wash with 10 mL water, elute with 10 mL 10 mM phosphoric acid in MeCN. Evaporate eluate to dryness at 50° under a stream of nitrogen and resuspend residue in 1 mL water. Centrifuge at 10000 g for 1 min, inject 100 μ L aliquot. Urine. Activate a Bond-Elut C18 cartridge with 1 volume MeOH and 2 volumes water. 1 mL Urine + 5 mL buffer, add to cartridge, wash with 10 mL MeCN, elute with 10 mL 10 mM phosphoric acid in MeCN. Evaporate eluate to dryness at 50° under a stream of nitrogen and resuspend residue in 1 mL water. Centrifuge at 10 000 g for 1 min, inject 100 μ L aliquot. (Buffer was 0.1 M citric acid:0.2 M Na₂HPO₄ 61.4:38.6 (McIlvaines buffer) containing 0.1 M disodium EDTA.)

HPLC VARIABLES**Guard column:** LiChrosorb RP-18**Column:** 150 \times 3.9 4 μ m Nova-Pak C18**Mobile phase:** MeCN:acetic acid:100 mM KH₂PO₄ 75:150:125 (serum) or 65:150:125 (urine)**Flow rate:** 1

Injection volume: 100

Detector: UV 340

CHROMATOGRAM

Retention time: 4

Limit of detection: 25 ng/mL

KEY WORDS

serum; SPE; protect from light with amber glassware

REFERENCE

Sheridan, M.E.; Clarke, G.S. Improved high-performance liquid chromatographic determination of doxycycline in serum and urine using solid-phase extraction columns. *J.Chromatogr.*, **1988**, *434*, 253-258

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 500 μ L Serum + 50 μ L 6% ascorbic acid in water + 50 μ L 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 μ L 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μ L mobile phase, mix, filter, keep in ice, inject a 20 μ L aliquot. Urine. 100 μ L Urine + 50 μ L 6% ascorbic acid in water + 50 μ L demeclocycline in MeOH/100 mM HCl + 400 μ L 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 μ L 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μ L mobile phase, mix, filter, keep in ice, inject a 20 μ L 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 \times 2.5 μ m Lichrosorb RP8

Mobile phase: MeCN:100 mM citric acid 24:76

Flow rate: 0.5

Injection volume: 20

Detector: UV 350

CHROMATOGRAM

Retention time: 9

Internal standard: demeclocycline (4)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, methacycline, oxytetracycline, tetracycline

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 10-100 μ g/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 \times 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: 40

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: cell suspensions

Sample preparation: 300 μL Cell suspension + 300 μL MeCN, vortex, centrifuge, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 \times 4 Nucleosil 100 5CN

Mobile phase: MeCN:THF:phosphate/citrate buffer 10:10:80

Injection volume: 10

Detector: UV 350

CHROMATOGRAM

Retention time: 2.1

REFERENCE

Kersten, A.; Poitschek, C.; Rauch, S.; Aberer, E. Effects of penicillin, ceftriaxone, and doxycycline on morphology of *Borrelia burgdorferi*. *Antimicrob.Agents Chemother.*, 1995, 39, 1127–1133

SAMPLE

Matrix: food

Sample preparation: Condition a 100 mg Baker 10 C18 SPE 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 the SPE 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 \times 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: 6
Limit of detection: 0.05 ppm

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline, tetracycline

KEY WORDS

honey; SPE

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: food

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 μ L 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 μ L aliquot.

HPLC VARIABLES

Column: 75 \times 4.6 3 μ m 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: 9
Limit of detection: 0.1 ppm

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline (demethylchlortetracycline), methacycline, minocycline, oxytetracycline, tetracycline

KEY WORDS

honey; 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: formulations

HPLC VARIABLES

Column: 250 × 4.6 5 μm Bakerbond phenylethyl

Mobile phase: MeOH:100 mM NaH₂PO₄ 70:30

Flow rate: 0.8

Detector: UV 280

CHROMATOGRAM

Retention time: 5.25 (doxycycline hyclate)

KEY WORDS

injections; saline; water; stability-indicating

REFERENCE

Stiles, M.L.; Allen, L.V., Jr.; Prince, S.J. Stability of various antibiotics kept in an insulated pouch during administration via portable infusion pump. *Am.J.Health-Syst.Pharm.*, **1995**, 52, 70–74

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' 0.56

OTHER SUBSTANCES

Simultaneous: anhydrotetracycline, chlortetracycline, demeclocycline, 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: 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 μ L 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 Na₂HPO₄, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m 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: 16.6

Limit of detection: 1.15 ng/mL

Limit of quantitation: 2.22 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline, 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. *JAOAC Int.*, **1993**, 76, 329-334

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 (ethylenedinitrilotetracetic 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 μ L 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 \times 100 5 μ m 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:** 26**Limit of quantitation:** 30 ng/g

OTHER SUBSTANCES**Simultaneous:** chlortetracycline, demethylchlortetracycline, methacycline, oxytetracycline, tetracycline

KEY WORDS

kidney; SPE

REFERENCEDegroot, 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:** Mince 0.1-0.3 g tissue with a scalpel and incubate at 37° with 0.5 mL water for 1 h. Add MeCN:85% phosphoric acid:water 20:2:78 (muscle, renal medulla, lung) or MeOH:MeCN:85% phosphoric acid:water 30:10:2:58 (renal cortex, liver) to a total volume of 1 mL, sonicate 30 min, filter (10 000 or 30 000 Da cutoff) by centrifuging at 2200 g for 30 min, inject 10-30 µL aliquot of filtrate.

HPLC VARIABLES**Column:** 220 × 2.1 Brownlee phenyl Spheri-5 MPLC cartridge**Mobile phase:** MeOH:MeCN:triethylamine:phosphoric acid:80 mM pH 2.4 sodium phosphate buffer 22.5:2.5:0.5:1.7:72.8**Column temperature:** 60**Flow rate:** 0.3-0.4**Injection volume:** 10-30**Detector:** UV 268; UV 345

CHROMATOGRAM**Retention time:** 6**Limit of detection:** <5-10 ng/g

KEY WORDS

cow; muscle; renal cortex; renal medulla; liver; lung

REFERENCERiond, J.L.; Hedeon, K.M.; Tyczkowska, K.; Riviere, J.E. Determination of doxycycline in bovine tissues and body fluids by high-performance liquid chromatography using photodiode array ultraviolet-visible detection. *J.Pharm.Sci.*, **1989**, *78*, 44–47

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Hoogmartens, J.; Khan, N.H.; Vanderhaeghe, H.; Van der Leeden, A..L.; Oosterbaan, M.; Veld-Tulp, G.L.; Plugge, W.; Van der Vlies, C.; Mialanne, D.; et al. A collaborative study of the analysis of doxycycline

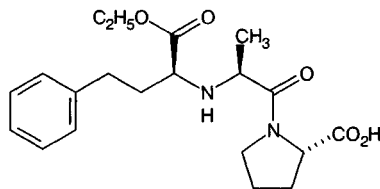
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- Böcker, R. Rapid analysis of doxycycline from biological samples by high-performance liquid chromatography. *J.Chromatogr.*, **1980**, *187*, 439–441 [whole blood; serum; tissue; mouse; liver]
- De Leenheer, A.P.; Nelis, H.J.C.F. Reversed-phase high-performance liquid chromatography of doxycycline. *J.Chromatogr.*, **1977**, *140*, 293–299

Enalapril

Molecular formula: C₂₀H₂₈N₂O₅

Molecular weight: 376.5

CAS Registry No.: 75847-73-3 (enalapril), 76095-16-4 (enalapril maleate)



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 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 12 μ m 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: 1.9

Internal standard: caffeine (4.8)

OTHER SUBSTANCES

Simultaneous: 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: Dissolve tablets in MeCN:1 mM pH 2 KH₂PO₄ 50:50, centrifuge, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb C8

Mobile phase: MeCN:buffer 35:65 (Buffer was 1 mM KH₂PO₄ adjusted to pH 2 with phosphoric acid.)

Column temperature: 40

Flow rate: 2.5

Injection volume: 50

Detector: UV 215

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: degradation products, enalaprilat, felodipine

KEY WORDS

tablets

REFERENCE

Qin, X.-Z.; DeMarco, J.; Ip, D.P. Simultaneous determination of enalapril, felodipine and their degradation products in the dosage formulation by reversed-phase high-performance liquid chromatography using a Spherisorb C₈ column. *J.Chromatogr.A*, **1995**, 707, 245–254

SAMPLE

Matrix: formulations

Sample preparation: Crush tablets, weigh out powder equivalent to 50 mg enalapril maleate, dissolve in 25 mL mobile phase, filter, dilute filtrate with an equal volume 1 mg/mL lisinopril in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4 ODS

Mobile phase: MeOH:water:phosphoric acid 75:25:0.1

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 8.0

Internal standard: lisinopril (13.2)

KEY WORDS

tablets

REFERENCE

Sane, R.T.; Vaidya, A.J.; Ghadge, J.K.; Jani, A.B.; Kotwal, S.K. Estimation of enalapril maleate in pharmaceutical dosage by HPLC. *Indian Drugs*, **1992**, 29, 244–245

SAMPLE

Matrix: formulations

Sample preparation: Crush tablet, mix with 10 mL mobile phase, filter, dilute with mobile phase to ca. 25 µg/mL, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Nucleosil C18

Mobile phase: MeCN:MeOH:water 50:25:25

Flow rate: 1

Injection volume: 100

Detector: UV 230

CHROMATOGRAM

Retention time: 2.3

KEY WORDS

tablets

REFERENCE

Rau, H.L.; Udupa, N.; Aroor, A.R. A new HPLC method for the estimation of enalapril maleate in tablets. *Indian Drugs*, **1991**, 29, 46–48

SAMPLE

Matrix: perfusate

Sample preparation: Hydrolyze with NaOH to enalaprilat.

HPLC VARIABLES

Column: 5 μ m Ultrasphere ODS

Mobile phase: MeCN:50 mM phosphate buffer 12:88, pH 3.2

Flow rate: 1

Detector: UV

CHROMATOGRAM

Limit of detection: 50 nM

KEY WORDS

rat

REFERENCE

Friedman, D.I.; Amidon, G.L. Passive and carrier-mediated intestinal absorption components of two angiotensin converting enzyme (ACE) inhibitor prodrugs in rats: enalapril and fosinopril. *Pharm.Res.*, **1989**, *6*, 1043-1047

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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: 6

OTHER SUBSTANCES

Simultaneous: benzepiril, captopril, cilazapril, quinapril, ramipril

REFERENCE

Barbato, F.; Morrica, P.; Quaglia, F. Analysis of ACE inhibitor drugs by high performance liquid chromatography. *Farmaco*, **1994**, *49*, 457-460

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak phenyl

Mobile phase: MeOH:water:85% phosphoric acid 60:40:0.05

Column temperature: 30-40

Detector: UV 215-220

OTHER SUBSTANCES

Also analyzed: lisinopril

REFERENCE

Ranadive, S.A.; Chen, A.X.; Serajuddin, A.T. Relative lipophilicities and structural-pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors. *Pharm.Res.*, **1992**, *9*, 1480-1486

Epoetin

Molecular formula: $C_{809}H_{1301}N_{229}O_{240}S_5$

Molecular weight: 30400 ± 400

CAS Registry No.: 113427-24-0 (α), 122312-54-3 (β)

SAMPLE

Matrix: blood

Sample preparation: Acidify serum or plasma with 8 volumes of ice-cold 0.1% trifluoroacetic acid, centrifuge at 10000 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: Whatman silica precolumn (Whatman No. 6561-403)

Column: two μ Bondapak C18 columns in series

Mobile phase: Gradient. MeCN:water:trifluoroacetic acid from 280:720:1 to 600:400:1 over 40 min, reequilibrate at 280:720:1 for 15 min before next injection.

Flow rate: 1.5

Detector: UV 280; collect 1.5 mL fractions and use bioassay

CHROMATOGRAM

Retention time: 32

Internal standard: coproporphyrin I (20)

KEY WORDS

serum; plasma; sheep; cow

REFERENCE

Congote, L.F. High-performance liquid chromatographic separation of serum erythropoietin and erythropoietin. *J.Chromatogr.*, **1984**, *310*, 396-400

SAMPLE

Matrix: formulations

Sample preparation: 300 μ L Liposome suspension + 90 μ L chloroform, centrifuge at 3000 rpm for 10 min, inject a 200 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: $250 \times 4.5 \mu\text{m}$ Vydac C4

Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 100:400:1. B was MeCN:water:trifluoroacetic acid 400:100:1. A:B 65:35 for 5 min, to 0:100 over 15 min, maintain at 0:100 for 2 min.

Flow rate: 1

Injection volume: 200

Detector: UV 214

CHROMATOGRAM

Retention time: 20

KEY WORDS

liposome suspensions

REFERENCE

Qi, X.-R.; Maitani, Y.; Shimoda, N.; Sakaguchi, K.; Nagai, T. Evaluation of liposomal erythropoietin prepared with reverse-phase evaporation vesicle method by subcutaneous administration in rats. *Chem.Pharm.Bull.*, **1995**, *43*, 295-299

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 600 × 7.5 10 μm TSK gel G3000SW (Toyo Soda)

Mobile phase: 20 mM pH 7.0 sodium citrate containing 100 mM NaCl

Flow rate: 1

Detector: UV 240

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

SEC

REFERENCE

Depaolis, A.M.; Advani, J.V.; Sharma, B.G. Characterization of erythropoietin dimerization. *J.Pharm.Sci.*, **1995**, *84*, 1280–1284

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 50 × 4.6 5 μm C4 214TP5405 (Vydac)

Mobile phase: Gradient. MeCN:0.06% trifluoroacetic acid 35:65 for 5 min, to 38:62 over 10 min, to 50:50 over 20 min, return to initial conditions over 5 min.

Flow rate: 1.5

Detector: UV 230

CHROMATOGRAM

Retention time: 23

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Depaolis, A.M.; Advani, J.V.; Sharma, B.G. Characterization of erythropoietin dimerization. *J.Pharm.Sci.*, **1995**, *84*, 1280–1284

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 50 × 4.6 YMC AP-800 C4 (Yamamura)

Mobile phase: Gradient. EtOH:buffer from 50:50 to 90:10 over 1 h (Buffer was 10 mM pH 7.0 Tris-HCl.)

Flow rate: 0.5

Detector: UV 280

CHROMATOGRAM

Retention time: 35

REFERENCE

Inoue, N.; Wada, M.; Takeuchi, M. An improved method for the purification of human erythropoietin with high *in vivo* activity from the urine of anemic patients. *Biol.Pharm.Bull.*, **1994**, *17*, 180–184

SAMPLE

Matrix: urine

Sample preparation: Concentrate urine by ultrafiltration-dialysis then chromatograph on Phenyl-Sepharose CL4B.

HPLC VARIABLES

Column: 75 × 7.5 Waters DEAE 5PW

Mobile phase: Gradient. A was 15 mM Tris adjusted to pH 8.6 with acetic acid. B was 15 mM Tris + 500 mM NaCl adjusted to pH 8.6 with acetic acid. A:B from 100:0 to 0:100 over 40 min.

Flow rate: 0.5

Detector: UV 280; bioassay

CHROMATOGRAM

Retention time: about 15

REFERENCE

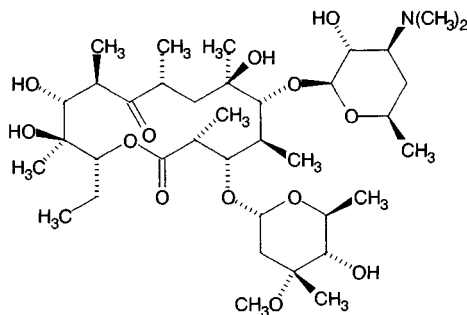
Lange, R.D.; Andrews, R.B.; Trent, D.J.; Reyniers, J.P.; Draganac, P.S.; Farkas, W.R. Preparation of purified erythropoietin by high performance liquid chromatography. *Blood Cells*, **1984**, *10*, 305–314

Erythromycin

Molecular formula: C₃₇H₆₇NO₁₃

Molecular weight: 733.9

CAS Registry No.: 114-07-8, 41342-53-4 (ethylsuccinate),
96128-89-1 (acistrate), 3521-62-8 (estolate),
304-63-2 (gluheptonate), 23067-13-2 (gluheptonate),
3847-29-8 (lactobionate), 134-36-1 (propionate),
643-22-1 (stearate), 84252-03-9 (stinoprate)



SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma or whole blood + 10 μ L 10 μ g/mL oleandomycin + 20 μ L saturated sodium carbonate + 1 mL diethyl ether, mix vigorously for 30 s, centrifuge at 6000 g for 2 min. Remove 750 μ L ether, evaporate to dryness under a stream of nitrogen at room temperature for 10 min, reconstitute residue in 50 μ L mobile phase, inject 20 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 4.6 5 μ m Asahi ODP-50G

Column: 150 \times 4.6 5 μ m Asahipak octadecyl polymer

Mobile phase: MeCN:50 mM pH 10.5 KH₂PO₄ 37:63

Flow rate: 1

Injection volume: 20

Detector: E, Shimadzu L-ECD-6A, glassy carbon electrode +0.72 V versus an Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 12.1

Internal standard: oleandomycin (5.7)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: clenbuterol, diltiazem, dipyrindamole, ketotifen, methacholine, orciprenaline, theophylline

KEY WORDS

plasma; whole blood

REFERENCE

Kato, Y.; Yokoyama, T.; Shimokawa, M.; Kudo, K.; Kabe, J.; Mohri, K. Determination of erythromycin in human plasma and whole blood by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1993**, *16*, 661-680

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 20 μ L of 100 μ g/mL oleandomycin phosphate in ethanol + 60 μ L saturated potassium carbonate (final pH 10), mix briefly, add 5 mL t-butyl methyl ether, shake in a reciprocating shaker for 15 min, centrifuge at 800 g for 5 min. Remove 4 mL of the upper ether layer and evaporate it to dryness under a stream of nitrogen at room temperature. Wash down tube with 200 μ L t-butyl methyl ether. Evaporate to dryness again, take up residue in 125 μ L mobile phase, centrifuge 30 s, inject aliquot.

HPLC VARIABLES

Guard column: Alltech Direct Connect packed with μ Bondapak C18

Column: 250 \times 4.6 5 μ m Ultrasphere C18

Mobile phase: MeCN:MeOH:buffer 42:10:48, final pH adjusted to 6.30-6.35 (Buffer was 100 mM sodium acetate adjusted to pH 5.0 with 100 mM acetic acid.)

Flow rate: 1.20

Injection volume: 20

Detector: E, ESA model 5100A, guard cell +0.95 V, detector 1 +0.65 V, detector 2 +0.85 V

CHROMATOGRAM

Retention time: 6

Internal standard: oleandomycin phosphate (4)

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: anhydroerythromycin, 2'-acetylerythromycin

KEY WORDS

plasma; mobile phase recirculated

REFERENCE

Laakso, S.; Scheinin, M.; Anttila, M. Determination of erythromycin base and 2'-acetylerythromycin in human plasma using high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1990**, *526*, 475-486

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 400 μ L 0.1 M NaOH to adjust pH to 10, mix 30 s, add 5 mL methyl t-butyl ether, vortex 1 min, centrifuge at 3000 rpm for 5 min, evaporate organic layer to dryness under a stream of nitrogen at 40°, dissolve residue in 150 μ L mobile phase, vortex 2 min, inject 10 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4 10 μ m Techopak T-15 C18

Mobile phase: MeCN:MeOH:THF:buffer 86:3:3:8 (Buffer was 75 mM sodium acetate adjusted to pH 4.1 with glacial acetic acid.)

Flow rate: 1.5

Injection volume: 10

Detector: E, ESA Model 5100A with ESA model 5020 guard cell, analytical cell + 0.70 V (I) +0.85 V (II), guard cell +0.90 V, 0.5 μ m carbon filters

CHROMATOGRAM

Retention time: 10

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma

REFERENCE

Kokkonen, P.; Haataja, H.; Välttilä, S. Determination of 2'-acetylerythromycin and erythromycin in plasma by HPLC using manual and robotic sample preparation. *Chromatographia*, **1987**, *24*, 680-682

SAMPLE

Matrix: blood, gastric juice

Sample preparation: Centrifuge plasma or gastric juice at 1200 g for 5 min. 500 μL Plasma or gastric juice + 500 μL pH 11 phosphate buffer (ionic strength $I=1.0$) + 50 μL 100 μM oleandomycin in MeCN + 5 mL hexane:2-butanol 80:20, shake for 15 min, centrifuge at 1200 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL mobile phase, vortex three times for 1 min, inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 μm Brownlee CN

Column: 100 \times 4.6 Hypersil C18 BDS base-deactivated

Mobile phase: MeCN:2.1 mM NaH_2PO_4 :27.1 mM Na_2HPO_4 40:30:30

Column temperature: 65

Flow rate: 1.2

Injection volume: 40

Detector: E, ESA Coulochem Model 5100A, Model 5020 guard cell before injector +1.0 V, model 5011 dual electrode analytical cell, screen electrode (detector 1) +0.65 V, sample electrode (detector 2) +0.85 V, ESA carbon filters before guard and analytical cells

CHROMATOGRAM

Retention time: 10.5

Internal standard: oleandomycin (5)

Limit of quantitation: 20 nM (plasma); 100 nM (gastric juice)

KEY WORDS

plasma; rugged; pharmacokinetics

REFERENCE

Toreson, H.; Eriksson, B.M. Determination of erythromycin in gastric juice and blood plasma by liquid chromatography and electrochemical detection. *J.Chromatogr.B*, **1995**, 673, 81–89

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Plasma. 2 mL Plasma + 20 μL 750 $\mu\text{g}/\text{mL}$ roxithromycin in MeCN + 5 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 5 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot. Urine. 1.5 mL Urine + 100 μL 750 $\mu\text{g}/\text{mL}$ roxithromycin in saturated K_2HPO_4 + 4 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 5 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot. Saliva. 1.5 mL Saliva + 100 μL 750 $\mu\text{g}/\text{mL}$ roxithromycin in saturated K_2HPO_4 + 4 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 15 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: MeCN:MeOH:56 mM sodium acetate buffer 50:4:56, final pH adjusted to 7.0 with glacial acetic acid

Flow rate: 1.1

Injection volume: 40

Detector: E, Waters M460, +0.9 V versus Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6.0 (erythromycin base), 7.1 (erythromycin B), 34.5 (erythromycin estolate), 35.5 (erythromycin ethylsuccinate)

Internal standard: roxithromycin (14.7)

Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES

Simultaneous: 4'-acetylerythromycin, 6-O-methylerythromycin

KEY WORDS

plasma

REFERENCE

Croteau, D.; Vallée, F.; Bergeron, M.G.; LeBel, M. High-performance liquid chromatographic assay of erythromycin and its esters using electrochemical detection. *J.Chromatogr.*, **1987**, *419*, 205–212

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Centrifuge urine at 2500 g for 5 min, inject a 100 μ L aliquot. Whole blood. 200 μ L Whole blood + 100 μ L 10% EDTA + 50 μ L 20 μ g/mL josamycin in MeOH:water 50:50, centrifuge to separate plasma. 200 μ L Plasma + 20 μ L saturated potassium carbonate + 1 mL MTBE, mix, centrifuge. Remove 800 μ L of the MTBE layer and evaporate it to dryness, reconstitute the residue in 200 μ L MeOH:water 50:50, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 8 μ m PLRP-S 1000 \AA (Polymer Labs)

Mobile phase: MeCN:t-butanol:200 mM pH 9.0 phosphate buffer:water 3:19:5:73

Column temperature: 70

Flow rate: 1.5

Injection volume: 100

Detector: F ex 365 em 450 following post-column extraction. The column effluent mixed with reagent pumped at 0.7 mL/min and this mixture flowed through a 1.5 m \times 0.5 mm ID stainless steel coil. The effluent from the coil mixed with chloroform pumped at 1.5 mL/min and this mixture flowed through a 1.5 m \times 0.5 mm ID stainless steel coil to a sandwich-type phase separator with a 40 μ L groove volume (Vrije Universiteit, Amsterdam). Part of the organic layer was separated and flowed through the detector at 0.5 mL/min. (Reagent was 5 μ M sodium 9,10-dimethoxyanthracene-2-sulfonate in 100 mM citric acid.)

CHROMATOGRAM

Retention time: 11

Internal standard: josamycin (28)

Limit of detection: 12.5 ng/mL (plasma); 50 ng/mL (urine)

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: midecamycin, troleandomycin

KEY WORDS

plasma; whole blood; post-column extraction

REFERENCE

Khan, K.; Paesen, J.; Roets, E.; Hoogmartens, J. Analysis of erythromycin A and its metabolites in biological samples by liquid chromatography with post-column ion-pair extraction. *J.Liq. Chromatogr.*, **1994**, *17*, 4195–4213

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute urine 1:2 with isotonic NaCl. 200 μ L Plasma or diluted urine + 100 μ L water + 600 μ L pH 9 phosphate buffer + 3 mL dichloromethane, shake for 10 min, centrifuge at 2000 g for 5 min. Remove 2.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L MeOH, vortex for 10 s, inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:83 mM ammonium acetate 55:22:23, pH adjusted to 7.5 with acetic acid

Flow rate: 1

Injection volume: 15

Detector: E, ESA Coulochem Model 5100A, Model 5020 guard cell 1.0 V (before injector), Model 5010 dual-electrode cell, screen electrode E1 + 0.7 V, sample electrode E2 + 0.9 V, 0.5 μ m ESA carbon filters placed before guard and analytical cells

CHROMATOGRAM

Retention time: 7.0

Internal standard: erythromycin

OTHER SUBSTANCES

Extracted: roxithromycin

Simultaneous: amitriptyline, clomipramine, disopyramide, erythromycin estolate, erythromycin ethylsuccinate, erythromycin stearate, imipramine, josamycin, lidocaine, spiramycin

KEY WORDS

plasma; erythromycin is IS

REFERENCE

Demotes-Mainaird, F.M.; Vinçon, G.A.; Jarry, C.H.; Albin, H.C. Micro-method for the determination of roxithromycin in human plasma and urine by high-performance liquid chromatography using electrochemical detection. *J. Chromatogr.*, **1989**, *490*, 115-123

SAMPLE

Matrix: blood, urine

Sample preparation: Prewash a 1 mL C18 Bondelut C18 SPE cartridge with 3 mL MeCN and 3 mL water. 1 mL Serum or urine + 0.25 mL (0.50 mL for urine) 6-12 μ g/mL oleandomycin phosphate in water, add 1 mL MeCN, vortex 1 min, centrifuge at 1600 g for 5 min, add to 8 mL water, load onto the SPE cartridge, wash with 5 mL water, wash with 5 mL MeCN:water 1:1, suck dry, elute with two 0.5 mL aliquots of MeCN:50 mM pH 6.30 phosphate buffer. Dry under vacuum in a rotary vacuum centrifuge, reconstitute in 20 μ L water, vortex 1 min, add 25 μ L MeCN, vortex 1 min, centrifuge at 1600 g for 1 min, inject 3-5 μ L aliquot of upper layer.

HPLC VARIABLES

Guard column: Waters Guard-Pak with Anatech 40-60 μ m glass beads

Column: 150 \times 3.9 Novapak C18

Mobile phase: MeCN:50 mM pH 6.30 phosphate buffer 30:70

Column temperature: 35

Flow rate: 1

Injection volume: 3-5

Detector: E, Metrohm 656 with a glassy carbon electrode, 1.15 V versus Ag/AgCl reference electrode; also UV at 200 nm with LOD 250-1000 ng/mL (*J. Pharm. Sci.* 1985, *74*, 1126-1128)

CHROMATOGRAM**Retention time:** 6.3**Internal standard:** oleandomycin phosphate (4.4)**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES**Simultaneous:** anhydroerythromycin

KEY WORDSserum; SPE; stability-indicating

REFERENCE

Stubbs, C.; Haigh, J.M.; Kanfer, I. A stability-indicating liquid chromatographic method for the analysis of erythromycin in stored biological fluids using amperometric detection. *J.Liq.Chromatogr.*, **1987**, *10*, 2547-2557

SAMPLE**Matrix:** formulations

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Bakerbond C18**Mobile phase:** MeCN:MeOH:200 mM ammonium acetate:water 45:10:10:25, pH 6.25**Flow rate:** 1**Detector:** UV 215

CHROMATOGRAM**Retention time:** 7.46 (erythromycin lactobionate)

KEY WORDSinjections; saline; water; stability-indicating

REFERENCE

Stiles, M.L.; Allen, L.V., Jr.; Prince, S.J. Stability of various antibiotics kept in an insulated pouch during administration via portable infusion pump. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 70-74

SAMPLE**Matrix:** formulations

Sample preparation: Weigh out material corresponding to ca. 250 mg erythromycin ethylsuccinate, add 10 mL acetone, sonicate 5 min, centrifuge at 2500 g for 5 min, dilute a 6 mL aliquot of supernatant to 10 mL with 200 mM pH 6.5 tetrabutylammonium hydrogen sulfate:200 mM pH 6.5 phosphate buffer:water 12.5:7.5:80.

HPLC VARIABLES**Column:** 250 × 4.6 10 μm RSil LL C18 (RSL-Bio-Rad)**Mobile phase:** MeCN:200 mM pH 6.5 tetrabutylammonium hydrogen sulfate (adjust pH with NaOH):200 mM pH 6.5 phosphate buffer:water 42.5:5:5:47.5**Column temperature:** 35**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 215

CHROMATOGRAM**Retention time:** 24 (erythromycin A ethylsuccinate), 8 (erythromycin A)

KEY WORDS

powders; tablets

REFERENCE

Cachet, T.; Lannoo, P.; Paesen, J.; Janssen, G.; Hoogmartens, J. Determination of erythromycin ethyl succinate by liquid chromatography. *J.Chromatogr.*, **1992**, *600*, 99–108

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax C8

Mobile phase: MeCN:200 mM pH 6.5 tetramethylammonium phosphate:200 mM pH 6.5 ammonium phosphate:water 35:20:5:40

Column temperature: 35

Flow rate: 1.5

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 12

KEY WORDS

better results with aged columns

REFERENCE

Cachet, T.; Quintens, I.; Roets, E.; Hoogmartens, J. Improved separation of erythromycin on aged reversed-phase columns. *J.Liq.Chromatogr.*, **1989**, *12*, 2171–2201

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Zierfels, G.; Petz, M. [Fluorimetric determination of erythromycin residues in foods of animal origin after derivatization with FMOC and HPLC separation]. *Z.Lebensm.Unters.Forsch.*, **1994**, *198*, 307–312

Janecek, M.; Quilliam, M.A.; Bailey, M.R.; North, D.H. Determination of erythromycin A by liquid chromatography and electrochemical detection, with application to salmon tissue. *J.Chromatogr.*, **1993**, *619*, 63–69 [electrochemical detection; fish; tissue; SPE; column temp 40; LOD 100 ng/g]

Paesen, J.; Calam, D.H.; Miller, J.H.McB.; Raiola, G.; Rozanski, A.; Silver, B.; Hoogmartens, J. Collaborative study of the analysis of erythromycin by liquid chromatography on wide-pore poly(styrene-divinylbenzene). *J.Liq.Chromatogr.*, **1993**, *16*, 1529–1544 [column temp 70; simultaneous impurities]

Cachet, T.; Quintens, I.; Paesen, J.; Roets, E.; Hoogmartens, J. Improved separation of erythromycin on aged reversed-phase columns. II. *J.Liq.Chromatogr.*, **1991**, *14*, 1203–1218

Paesen, J.; Roets, E.; Hoogmartens, J. Liquid chromatography of erythromycin A and related substances on poly(styrene-divinylbenzene). *Chromatographia*, **1991**, *32*, 162–166

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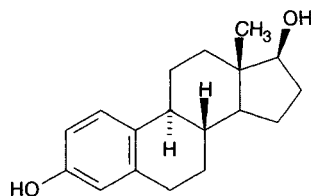
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- Tsuji, K.; Kane, M.P. Improved high-pressure liquid chromatographic method for the analysis of erythromycin in solid dosage forms. *J.Pharm.Sci.*, **1982**, *71*, 1160–1164

Estradiol

Molecular formula: C₁₈H₂₄O₂

Molecular weight: 272.4

CAS Registry No.: 50-28-2, 113-38-2 (dipropionate),
979-32-8 (valerate), 57-91-0 (α -estradiol),
50-50-0 (benzoate), 313-06-4 (cypionate),
4956-37-0 (enanthate), 3571-53-7 (undecylenate)



SAMPLE

Matrix: blood

Sample preparation: Inject 10 μ L plasma into MeCN pumped at 0.2 mL/min so that the precipitated proteins are removed by 0.5 and 0.2 μ m filters in series. Switch the MeCN containing sample into the mobile phase and allow it to pass onto the analytical column, elute the analytical column in the usual way with mobile phase. Remove the filter unit from the circuit and back-flush it to waste with 100 mM sodium dodecyl sulfate at 2 mL/min, equilibrate filters with MeCN for 5 min before next injection.

HPLC VARIABLES

Guard column: 20 mm Brownlee C18

Column: 250 \times 4.6 5 μ m Ultrasphere C18

Mobile phase: MeCN:water 33:67

Flow rate: 1

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 25.8

OTHER SUBSTANCES

Simultaneous: equilin, estrone

KEY WORDS

plasma; dog

REFERENCE

Asafu-Adjaye, E.B.; Su, S.Y.; Shiu, G.K. Switching-valve-filter technique for the direct injection and analysis of drugs in plasma using high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *652*, 35-42

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 500 μ L 10 M NaOH, shake on a slow rotatory mixer for 5 min, add 5 mL diethyl ether, rotomix 10 min, centrifuge at 700 g for 5 min, repeat extraction. Combine organic layers, evaporate to dryness under a stream of nitrogen at 37°, dissolve in 250 μ L mobile phase, inject aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Novapack C18

Mobile phase: MeCN:MeOH:buffer 35:15:50 (Buffer was 50 mM KH₂PO₄ adjusted to pH 3.6 with phosphoric acid.)

Flow rate: 1.6

Injection volume: 50

Detector: E, Waters Model 464 pulsed electrochemical detector, + 1 V versus Ag/AgCl

CHROMATOGRAM

Retention time: 2.44

Limit of detection: 50 pg/mL

OTHER SUBSTANCES

Simultaneous: estriol, estrone, ethinylestradiol, heparin

Noninterfering: pentobarbital

KEY WORDS

plasma; rabbit

REFERENCE

Fernández, N.; Garcia, J.J.; Diez, M.J.; Terán, M.T.; Sierra, M. Rapid high-performance liquid chromatographic assay of ethinyloestradiol in rabbit plasma. *J.Chromatogr.*, **1993**, *619*, 143–147

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 500 μ L water + 100 μ L 10 μ g/mL 3,7-dimethoxyflavone in EtOH + 8 mL diethyl ether, shake, centrifuge at 4° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L MeOH:water 40:60, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 3 μ m NS-Gel C18

Mobile phase: Gradient. MeOH:water from 40:60 to 55:45, maintain at 55:45 for 24 min, to 80:20 over 25 min.

Column temperature: 50

Flow rate: 1

Injection volume: 50

Detector: UV 210; UV 240

CHROMATOGRAM

Retention time: 30.74

Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES

Extracted: aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estrone, hydrocortisone, 17-hydroxyprogesterone, pregnenolone, progesterone

KEY WORDS

serum

REFERENCE

Ueshiba, H.; Segawa, M.; Hayashi, T.; Miyachi, Y.; Irie, M. Serum profiles of steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method. *Clin.Chem.*, **1991**, *37*, 1329–1333

SAMPLE

Matrix: blood

Sample preparation: Extract 1 mL serum twice with 5 volumes ether by vortexing for 2 min, evaporate extracts to dryness under a stream of nitrogen at 35°, reconstitute in 100 µL MeOH.

HPLC VARIABLES

Column: 240 × 4.5 Bio-Rad ODS-5S

Mobile phase: Gradient. MeOH:MeCN:water at 20:60:20 for 3 min then to 5:85:10 over 26 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

OTHER SUBSTANCES

Simultaneous: androstenedione, progesterone, testosterone

KEY WORDS

serum

REFERENCE

Yu, F.H.; Yun, Y.W.; Yuen, B.H.; Moon, Y.S. Effects of hydroxyflutamide on rats treated with a superovulatory dose of pregnant mare serum gonadotropin. *Can.J.Physiol.Pharmacol.*, **1991**, 69, 185–190

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 1 mL 500 mM pH 7 phosphate buffer + 12 mL hexane:ethyl acetate 70:30, extract. Remove a 10 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 µL mobile phase, inject a 20-50 µL aliquot. (Hydrolyze 500 µL plasma by adding 500 µL 200 mM pH 5 acetate buffer and 100 µL beef liver β-glucuronidase (Sigma) or 10 µL β-glucuronidase/sulfatase (Glusulase), heat at 37° overnight, add 1 mL 500 mM pH 7 phosphate buffer + 12 mL hexane:ethyl acetate 70:30, extract. Remove a 10 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 µL mobile phase, inject a 20-50 µL aliquot.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Partisil 5/25 silica gel

Mobile phase: Hexane:EtOH 92.5:7.5

Flow rate: 1.5

Injection volume: 20-50

Detector: F ex 195 em 250 (cut-off filter)

CHROMATOGRAM

Retention time: 8.9

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, estramustine, estromustine, estrone

KEY WORDS

plasma; rat; dog; human; pharmacokinetics; normal phase

REFERENCE

Dixon, R.; Brooks, M.; Gill, G. Estramustine phosphate: Plasma concentrations of its metabolites following oral administration to man, rat and dog. *Res.Commun.Chem.Pathol.Pharmacol.*, **1980**, 27, 17–29

SAMPLE

Matrix: blood, tissue

Sample preparation: 1 mL Blood or brain + 1 mL 50 mM ammonium acetate buffer, homogenize (Polytron PT-1200C), add 4 mL MeCN, vortex, add 1 mL concentrated brine, allow to stand at -5° for 1 h. Remove the organic phase and centrifuge it at 3000 g, filter, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C8

Mobile phase: MeCN:50 mM pH 6.8 ammonium acetate 52:48 containing 10 mM tetraethylammonium perchlorate

Flow rate: 1

Detector: UV 280

CHROMATOGRAM

Retention time: 3.8

Limit of detection: 133 ng/g

KEY WORDS

whole blood; rat; brain

REFERENCE

Brewster, M.E.; Druzgala, P.J.; Anderson, W.R.; Huang, M.-J.; Bodor, N.; Pop, E. Efficacy of a 3-substituted versus 17-substituted chemical delivery system for estradiol brain targeting. *J.Pharm.Sci.*, 1995, 84, 38-43

SAMPLE

Matrix: culture medium

Sample preparation: Extract culture medium twice with 2 volumes of ether, combine the extracts and evaporate them to dryness, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Techopak 10 C18 (HPLC Technology)

Mobile phase: MeOH:0.5% pH 3.0 (NH₄)H₂PO₄ 62:39

Flow rate: 0.7

Detector: UV 280; Radioactivity

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Extracted: estrone

KEY WORDS

tritium labeled

REFERENCE

Wild, M.J.; Rudland, P.S.; Back, D.J. Metabolism of the oral contraceptive steroids ethynylestradiol and norgestimate by normal (Huma 7) and malignant (MCF-7 and ZR-75-1) human breast cells in culture. *J.Steroid Biochem.Mol.Biol.*, 1991, 39, 535-543

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 5 mL ethyl acetate, vortex, centrifuge at 2000 g for 8 min, remove organic phase, repeat extraction. Combine the organic layers

and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:water 50:50, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μm Ultracarb 30 ODS (Phenomenex)

Mobile phase: Gradient. MeCN:0.1% acetic acid in MeOH:0.1% acetic acid in water 16:12:72 for 3 min, to 20:21:59 over 25 min (Waters no. 3 convex gradient), to 24:23:53 over 10 min (linear), to 55:24:21 over 10 min (linear), to 92:5:3 over 1 min, maintain at 92:5:3 for 7 min, return to initial conditions over 15 min.

Flow rate: 1.2

Injection volume: 50

Detector: UV 280

CHROMATOGRAM

Retention time: 46

OTHER SUBSTANCES

Extracted: metabolites, estrone

KEY WORDS

rat

REFERENCE

Suchar, L.A.; Chang, R.L.; Rosen, R.T.; Lech, J.; Conney, A.H. High-performance liquid chromatography separation of hydroxylated estradiol metabolites: Formation of estradiol metabolites by liver microsomes from male and female rats. *J.Pharmacol.Exp.Ther.*, **1995**, 272, 197–206

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Human placental microsome suspension + 1 mL dichloromethane, extract, centrifuge, remove organic layer and evaporate it under vacuum, dissolve residue in 30 μL MeCN:water 50:50, centrifuge for 3 min, inject supernatant. After each run wash column with MeCN for 1 min, re-equilibrate for 1 min.

HPLC VARIABLES

Column: 50 \times 4.6 3 μm Spherisorb ODS-2

Mobile phase: MeCN:water 50:50

Column temperature: 60

Flow rate: 2

Injection volume: 30

Detector: UV 200

CHROMATOGRAM

Retention time: 0.6

Limit of detection: <0.1 nmol/mL

OTHER SUBSTANCES

Simultaneous: androstenedione, estrone, testosterone

KEY WORDS

human; placenta

REFERENCE

Taniguchi, H.; Feldmann, H.R.; Kaufmann, M.; Pyerin, W. Fast liquid chromatographic assay of androgen aromatase activity. *Anal.Biochem.*, **1989**, 181, 167–171

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 × 4.6 5 μm Ultrasphere**Mobile phase:** MeCN:EtOH:water 54:1:45**Flow rate:** 1.5**Detector:** UV 270

CHROMATOGRAM**Retention time:** 2.4 (17β-estradiol)

REFERENCE

Fridriksdottir, H.; Loftsson, T.; Gudmundsson, J.A.; Bjarnason, G.J.; Kjeld, M.; Thorsteinsson, T. Design and in vivo testing of 17β-estradiol-HPβCD sublingual tablets. *Pharmazie*, **1996**, *51*, 39–42

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Nucleosil phenyl**Mobile phase:** Gradient. Carbon dioxide:MeOH from 98:2 to 78:22 over 40 min.**Column temperature:** 50**Flow rate:** 2**Detector:** UV

CHROMATOGRAM**Retention time:** 10.3

OTHER SUBSTANCES**Simultaneous:** other steroids, estriol, hydrocortisone, hydroxyprogesterone, norethisterone, testosterone

KEY WORDSSFC; 200 bar

REFERENCE

Hanson, M. Aspects of retention behaviour of steroids in packed column supercritical fluid chromatography. *Chromatographia*, **1995**, *40*, 58–68

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare an aqueous solution, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 3.5 μm Zorbax SB C18**Mobile phase:** MeCN:MeOH:buffer 15:45:40 (Buffer was 10 mM KH₂PO₄ and 50 mM tetrabutylammonium chloride, pH adjusted to 3.0 with 1 M HCl.)**Flow rate:** 0.9**Injection volume:** 20**Detector:** UV 220

CHROMATOGRAM**Retention time:** 9.6 (17β-estradiol), 6.9 (17β-estradiol-3-phosphate)

OTHER SUBSTANCES

Simultaneous: estriol, estrone, estrone-3-phosphate

KEY WORDS

stability-indicating (for 17 β -estradiol-3-phosphate)

REFERENCE

Miller, R.B.; Chen, C. A stability-indicating HPLC method for the determination of 17 β -estradiol-3-phosphate in an ophthalmic solution. *Chromatographia*, **1995**, *40*, 204–206

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 \times 4.5 μ m 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: ELSD (Sédex 55, Sédéré)

CHROMATOGRAM

Retention time: 18.22

OTHER SUBSTANCES

Simultaneous: acetylcholine, cholesterol, choline, cortisone, dextrose, glycine, phenylalanine, 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 \times 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, benzphenetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphe-

nesin, 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, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, 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, isocarboxtyril, 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, mephen-
 termine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylpyrrolon, metoprolol, mibolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, nor-
 epinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phenidimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrihydione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albu-
 terol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelethamine, triproilidine, 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 280

CHROMATOGRAM

Retention time: k' 3.462

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone (UV 240), medroxyprogesterone acetate (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone (UV 240), methylprednisolone acetate (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone (UV 240), prednisolone acetate (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 solution in MeOH.

HPLC VARIABLES

Column: Radial-PAK μ Bondapak C18

Mobile phase: MeCN:water 50:50

Flow rate: 2

Injection volume: 100

Detector: UV 254; UV 214

CHROMATOGRAM

Retention time: 5.6

OTHER SUBSTANCES

Simultaneous: estriol, estrone, progesterone

Interfering: estradiol, testosterone

REFERENCE

Ercoc, F.U.; Özsar, S.; Güven, B.; Kalkandelen, G.; Ugrar, E. High-performance liquid chromatographic analysis of steroid hormones. *J.Chromatogr.Sci.*, **1989**, *27*, 86–90

SAMPLE

Matrix: solutions

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

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 80:1.5:0.5:18

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 0.41 (estradiol), k' 3.53 (estradiol benzoate), k' 7.45 (estradiol cypionate), k' 3.49 (estradiol valerate)

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 solution in EtOH, inject an aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 5 μm Spherisorb S5-ODS**Mobile phase:** Gradient. MeOH:20 mM ammonium sulfate from 30:70 to 100:0 over 35 min**Column temperature:** 45**Flow rate:** 1**Injection volume:** 50**Detector:** F ex 214 em 340 (cut-off); UV 280

CHROMATOGRAM**Retention time:** 13 (17β-estradiol-3-sulfate), 23 (17β-estradiol)

OTHER SUBSTANCES**Simultaneous:** estriol, estriol-3-sulfate, estrone, estrone-3-sulfate

REFERENCESimonian, M.H.; Capp, M.W. Reversed-phase high-performance liquid chromatography of steroid 3-sulfates and the corresponding unconjugated steroids. *J.Chromatogr.*, **1984**, *287*, 97–104

SAMPLE**Matrix:** tissue**Sample preparation:** Incubate endometrial tissue with buffer, remove tissue, extract medium twice with 2 volumes of diethyl ether, evaporate to dryness, reconstitute in a small volume of MeOH, inject an aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 Technopak 10 C18**Mobile phase:** MeOH:0.5% pH 3.0 (NH₄)₂PO₄ 62:38**Flow rate:** 0.7**Detector:** UV 280

CHROMATOGRAM**Retention time:** 25

OTHER SUBSTANCES**Simultaneous:** estrone

KEY WORDS

endometrial tissue

REFERENCEWild, M.J.; Rudland, P.S.; Back, D.J. Metabolism of the oral contraceptive steroids ethynylestradiol, norgestimate and 3-ketodesogestrel by a human endometrial cancer cell line (HEC-1A) and endometrial tissue *in vitro*. *J.Steroid Biochem.Mol.Biol.*, **1993**, *45*, 407–420

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 Carboxypack column, wash column with 2 mL MeOH:water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, wash with 1 mL MeOH, 1 mL 1 M HCl, elute with 2 mL 30 mM HCl in MeCN:MeOH 20:80. Evaporate eluate to dryness with nitrogen at 40°, take up in 100 μ L MeCN:MeOH:THF:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 22:8:13:57, inject 40 μ L aliquot

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Supelguard LC-18

Column: 250 \times 4.6 5 μ m Supelco C18

Mobile phase: MeCN:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 46:54

Flow rate: 1.2

Injection volume: 40

Detector: F ex 280 em 308

CHROMATOGRAM

Retention time: 7

Limit of detection: 1 ng/g

KEY WORDS

muscle; liver; chicken; ox; cow

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: urine

Sample preparation: 50 mL Urine + 7 mL concentrated HCl, heat at 90° for 1 h, add 10 μ L 1 mg/mL 4-phenylphenol in MeOH, extract 3 times with 10 mL diethyl ether, combine organic phases, wash twice with 20 mL portions of pH 10.5 $\text{NaHCO}_3/\text{NaOH}$ buffer, wash with 20 mL water, dry over 5 g anhydrous sodium sulfate. Filter, evaporate under reduced pressure almost to dryness, take up residue in 1 mL mobile phase, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 Beckman ODS

Mobile phase: MeCN:water 25:75 containing 14 mM β -cyclodextrin

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: UV 280; F ex 280 em 312

CHROMATOGRAM

Retention time: 8.1

Internal standard: 4-phenylphenol

Limit of detection: 1-3 ng/mL

OTHER SUBSTANCES

Simultaneous: estriol, estrone

REFERENCE

Lamparczyk, H.; Zarzycki, P.K.; Nowakowska, J.; Ochocka, R.J. Application of β -cyclodextrin for the analysis of estrogenic steroids in human urine by high-performance liquid chromatography. *Chromatographia*, **1994**, *38*, 168–172

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Liu, P.; Higuchi, W.I.; Ghanem, A.-H.; Good, W.R. Transport of β -estradiol in freshly excised human skin in vitro: Diffusion and metabolism in each skin layer. *Pharm.Res.*, **1994**, *11*, 1777–1784 [perfusate]

Patel, J.U.; Pranker, R.J.; Sloan, K.B. A prodrug approach to increasing the oral potency of a phenolic drug. 1. Synthesis, characterization, and stability of an O-(imidomethyl) derivative of 17β -estradiol. *J.Pharm.Sci.*, **1994**, *83*, 1477–1481

Sheikh, S.U.; Touchstone, J.C. Determination of free estriol in amniotic fluid by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1994**, *17*, 3813–3820 [amniotic fluid; plasma; column temp 20; also estriol; SPE]

Chong, K.Y.; Khoo, T.H.; Koo, F.S.; Ong, C.P.; Li, S.F.Y.; Lee, H.K.; Venkatesh, B.; Tan, C.H. Optimization of the high-performance liquid chromatographic separation of steroids by the overlapping resolution mapping procedure. *J.Liq.Chromatogr.*, **1991**, *14*, 2445–2455 [simultaneous androstenedione, 5α -dihydrotestosterone, $17\alpha,20\beta$ -dihydroxyprogesterone, 17α -hydroxyprogesterone, 11β -hydroxytestosterone, 11-ketotestosterone, progesterone, testosterone]

Hines, G.A.; Watts, S.A.; Sower, S.A.; Walker, C.W. Sex steroid extraction from echinoderm tissues. *J.Liq.Chromatogr.*, **1990**, *13*, 2489–2498 [testis; ovary; pyloric caecal; also progesterone, testosterone; SPE; sea star; sea urchin; radiolabeled compounds]

Jiang, L.-X.; Wang, Z.-J.; Matlin, S.A. HPLC analysis of injectable contraceptive preparation containing norethisterone enanthate and estradiol valerate. *J.Liq.Chromatogr.*, **1990**, *13*, 3473–3479 [simultaneous benzyl benzoate, estradiol valerate, norethisterone enanthate; column temp 15]

Formento, J.L.; Moll, J.L.; Francoual, M.; Krebs, B.P.; Milano, G.; Renee, N.; Khater, R.; Frenay, M.; Namer, M. HPLC micromethod for simultaneous measurement of estradiol, progesterone, androgen and glucocorticoid receptor levels. Application to breast cancer biopsies. *Eur.J.Cancer Clin.Oncol.*, **1987**, *23*, 1307–1314

Sheikh, S.U.; Touchstone, J. HPLC of steroids in non-aqueous mobile phase at subambient temperature. *J.Liq.Chromatogr.*, **1987**, *10*, 2489–2496 [column temp -50; also cortisone, desoxycorticosterone, estrone; hydrocortisone]

Carignan, G.; Lodge, B.A.; Skakum, W. Simultaneous analysis of estradiol dienanthate, estradiol 3-benzoate and testosterone enanthate benzilic acid hydrazone in oily formulations by gradient HPLC. *J.Liq.Chromatogr.*, **1985**, *8*, 2567–2577 [formulations; oils; gradient; simultaneous estradiol dienanthate, estradiol 3-benzoate, testosterone enanthate benzilic acid hydrazone; 1,2,4,5-tetrachlorobenzene (IS)]

Hayashi, N.; Hayata, K.; Sekiba, K. Rapid and simultaneous measurement of estrone, estradiol, estriol and estetrol in serum by high performance liquid chromatography with electrochemical detection. *Acta Med.Okayama.*, **1985**, *39*, 143–153

Batra, S.K.; Saumande, J. High performance liquid chromatographic separation of estradiol- 17α and- 17β in biological fluids; Application to plasma, milk and urine of cows. *J.Liq.Chromatogr.*, **1984**, *7*, 2431–2446 [plasma; milk; urine; cow; radioimmunoassay detection; LOD 100 pg/mL]

Carignan, G.; Lodge, B.A.; Skakum, W. High-performance liquid chromatographic analysis of estradiol valerate-testosterone enanthate in oily formulations. *J.Chromatogr.*, **1984**, *301*, 292–296

Fast, D.M.; Culbreth, P.H.; Sampson, E.J. Multivariate and univariate optimization studies of liquid-chromatographic separation of steroid mixtures. *Clin.Chem.*, **1982**, *28*, 444–448 [also estriol, hydrocortisone, progesterone, testosterone]

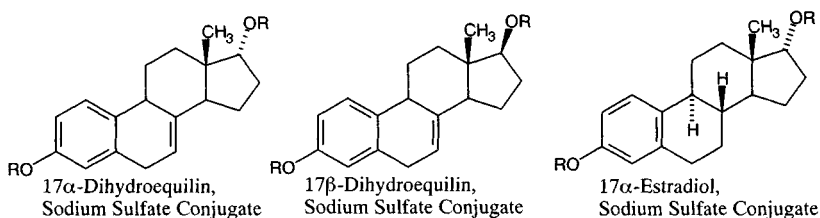
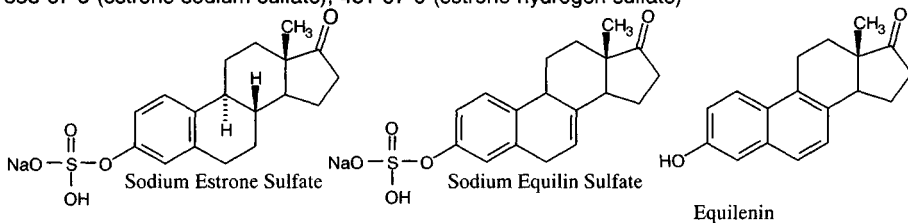
Kessler, M.J. A rapid high performance liquid chromatography system for the separation of gonadal steroids. *J.Liq.Chromatogr.*, **1982**, *5*, 125–139 [simultaneous androstandiol, androstenedione, androsthenone, dehydroepiandrostenone, dihydrotestosterone, estriol, hydroxyprogesterone, hydroxytestosterone, pregnenolone, progesterone, testosterone]

Estrogens, Conjugated

Molecular formula: $C_{18}H_{18}O_2$ (equilenin), $C_{18}H_{22}O_2$ (17α -dihydroequilin), $C_{18}H_{20}O_2$ (equilin), $C_{18}H_{22}O_2$ (estrone), $C_{18}H_{24}O_2$ (estradiol)

Molecular weight: 266.3 (equilenin), 272.4 (estradiol), 268.3 (equilin), 270.4 (17α -dihydroequilin), 270.4 (estrone)

CAS Registry No.: 474-86-2 (equilin), 50-28-2 (estradiol), 57-91-0 (α -estradiol), 517-09-9 (equilenin), 53-16-7 (estrone), 338-67-5 (estrone sodium sulfate), 481-97-0 (estrone hydrogen sulfate)



R = H, HSO_3Na

Conjugated Estrogens
(see Preface)

SAMPLE

Matrix: blood

Sample preparation: Add 0.1 (rabbit) or 1 (rat, monkey) plasma to 1 mL 100 mM pH 5.0 acetate buffer and 50 μ L Glusulase (from Helix Pomatia, contains 10000 U/mL sulfatase and 90000 U/mL β -glucuronidase, DuPont), heat at 37° for 1 h, cool to room temperature, add 15 mL diethyl ether, shake mechanically at high speed for 10 min, centrifuge at 3033 g for 10 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μ L mobile phase, filter (0.45 μ m), inject a 150 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 150 \times 3.5 μ m C6 (Column Engineering, Ontario CA)

Mobile phase: MeCN:MeOH:50 mM pH 3.5 ammonium acetate 27:8:65

Flow rate: 0.35

Injection volume: 150

Detector: F ex 210 em 370

CHROMATOGRAM

Retention time: 19.5 (17α -dihydroequilenin)

Internal standard: 14β -equilenin (24)

Limit of quantitation: 2.5 ng/mL (rat), 5 ng/mL (rabbit, monkey)

OTHER SUBSTANCES

Noninterfering: equilenin, 17α -dihydroequilenin

KEY WORDS

plasma; rat; rabbit; monkey; pharmacokinetics

REFERENCE

Chandrasekaran, A.; Osman, M.; Adelman, S.J.; Warsheski, J.; Scatina, J.; Sisenwine, S.F. Determination of 17 α -dihydroequilenin in rat, rabbit and monkey plasma by high-performance liquid chromatography with fluorimetric detection. *J.Chromatogr.B*, **1996**, 676, 69–75

SAMPLE

Matrix: blood

Sample preparation: 10 μ L Plasma is injected into MeCN pumped at 0.2 mL/min, precipitated proteins are removed by 0.5 and 0.2 μ m filters in series, MeCN containing sample is switched into mobile phase allowed to pass onto analytical column. Next filter unit is switched out of circuit and back-flushed to waste with 100 mM sodium dodecyl sulfate at 2 mL/min, analytical column is eluted in normal fashion with mobile phase. Equilibrate filters with MeCN for 5 min before next injection.

HPLC VARIABLES

Guard column: 20 mm Brownlee C18

Column: 250 \times 4.6 5 μ m Ultrasphere C18

Flow rate: 1

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 25.8 (estradiol), 36.6 (equilin), 42.6 (estrone)

KEY WORDS

plasma; dog

REFERENCE

Asafu-Adjaye, E.B.; Su, S.Y.; Shiu, G.K. Switching-valve-filter technique for the direct injection and analysis of drugs in plasma using high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, 652, 35–42

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 10 μ L 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 μ L MeCN. Add 10 μ L reagent 1, add 10 μ L reagent 2, heat at 50° for 15 min, cool to room temperature, add 100 μ L water, add 200 μ L 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 μ L by evaporation at 40° under reduced pressure, inject 20 μ L 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.)

HPLC VARIABLES

Guard column: 50 \times 4 5 μ m Wakosil 5C18

Column: 300 \times 4 5 μ m Wakosil 5C18

Mobile phase: MeOH:water 90:10

Flow rate: 0.7

Injection volume: 20

Detector: F ex 336 em 440

CHROMATOGRAM

Retention time: 10.5 (estriol), 15.4 (ethynylestradiol), 16.5 (equilin), 16.5 (equilenin), 17.2 (estrone), 18.2 (estradiol), 19.2 (estetrol), 28.1 (4-hydroxyestradiol), 35.5 (2-hydroxyestradiol)

Internal standard: sec-butyl p-hydroxybenzoate (14.3)

Limit of detection: 1-2 pg/mL

KEY WORDS

plasma; equilin and equilenin not resolved

REFERENCE

Katayama, M.; Taniguchi, H. Determination of estrogens in plasma by high-performance liquid chromatography after pre-column derivatization with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole. *J.Chromatogr.*, **1993**, 616, 317-322

SAMPLE

Matrix: blood

Sample preparation: 0.5 mL Serum + 0.5 mL MeCN:water 1:1, vortex 15 s, add 3 mL MeCN, shake 1 min, centrifuge at 1800 rpm for 10 min. Remove supernatant and dry it under a stream of nitrogen at 55°, add 2 mL MeCN:MeOH 1:1, vortex 15 s, centrifuge at 1800 rpm for 10 min. Remove supernatant and dry it under a stream of nitrogen at 55°. Reconstitute in 200 µL MeCN:water 1:1.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Beckman ODS

Mobile phase: A 2% tetrabutylammonium hydroxide adjusted to pH 3 with phosphoric acid.

B MeCN:water 33:67 A:B was 6.5:93.5

Flow rate: 0.8

Injection volume: 20

Detector: UV 210; F ex 280 em 312

CHROMATOGRAM

Retention time: 53 (estrone), 48 (equilin), 41 (estrone sulfate), 38 (equilin sulfate), 32 (estradiol), 26 (17- α -dihydroequilin sulfate)

Limit of detection: 10-100 ng/mL

KEY WORDS

serum

REFERENCE

Su, S.Y.; Shiu, G.K.; Simmons, J.; Viswanathan, C.T.; Skelly, J.P. High performance liquid chromatographic analysis of six conjugated and unconjugated estrogens in serum. *Biomed.Chromatogr.*, **1992**, 6, 265-268

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.

HPLC VARIABLES

Column: 300 × 4 10 µm µBondapak C18

Mobile phase: MeOH:buffer 65:35 (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: 5.3
Internal standard: equilenin
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: betamethasone, deoxycortisol, dexamethasone, hydrocortisone, prednisone, triamcinolone

KEY WORDS

Anal.Abs. 1982, 43, 4D182; plasma=uilenin is IS

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: cells

Sample preparation: Homogenize (glass-glass homogenizer) cells in medium, centrifuge at 1000 g for 5 min. Add 3 mL homogenate to 10 mL diethyl ether:acetone 90:10, mix thoroughly for a few s, let stand at 4° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in three 500 µL portions of acetone, evaporate to dryness under a stream of nitrogen, reconstitute with 30 µL MeCN, inject a 20 µL aliquot.

HPLC VARIABLES

Column: Ultrasphere ODS
Mobile phase: MeCN:10 mM citric acid 40:60
Column temperature: 20 ± 1
Flow rate: 1
Injection volume: 20
Detector: UV 280; Radioactivity

CHROMATOGRAM

Retention time: 14 (17β-estradiol), 16 (equilin), 19 (estrone)

KEY WORDS

tritium labeled; ¹⁴C labeled

REFERENCE

Castagnetta, L.A.; Granata, O.M.; Lo Casto, M.; Calabro, M.; Arcuri, F.; Carruba, G. Simple approach to measure metabolic pathways of steroids in living cells. *J.Chromatogr.*, **1991**, 572, 25-39

SAMPLE

Matrix: formulations

Sample preparation: Dissolve a quantity equivalent to about 25 mg of conjugated estrogens in 20 mL MeOH, add 20 mL water, add 4 mL concentrated HCl, add several boiling chips, boil for 5 min, cool to room temperature, add 2.5 mL 0.5 mg/mL estriol in MeOH, extract twice with 10 mL and once with 5 mL portions of chloroform, combine extracts, wash with 5 mL water, pass through 1 g anhydrous sodium sulfate, evaporate to dryness under nitrogen, dissolve residue in 25 mL MeOH:water 1:1, inject aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 3 μm Nucleosil C18**Mobile phase:** MeOH:water:isopropanol:dichloromethane 45:42.5:7.5:5**Flow rate:** 0.7**Injection volume:** 20**Detector:** UV 280; E, Laboratorni Pristroje ADLC 2 detector, carbon fiber working electrode, stainless steel counter electrode, 1.1 V vs Ag/AgCl reference electrode, 0.6% Na₂HPO₄·12H₂O added to mobile phase which was adjusted to pH 6.0-6.05 with acetic acid

CHROMATOGRAM**Retention time:** 23.18 (estrone), 20.59 (equilin), 18.59 (equilenin), 15.14 (17α-estradiol), 13.36 (17α-dihydroequilin), 11.85 (17α-dihydroequilenin)**Internal standard:** estriol (6.44)**Limit of detection:** 2-4 μg/mL

KEY WORDS

tablets; capsules

REFERENCENovakovic, J.; Tvrzická, E.; Pacáková, V. High-performance liquid chromatographic determination of equine estrogens with ultraviolet absorbance and electrochemical detection. *J.Chromatogr.A*, **1994**, *678*, 359-363

SAMPLE**Matrix:** formulations**Sample preparation:** Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 150 × 3.9 4 μm NovaPak C18**Mobile phase:** MeOH:25 mM KH₂PO₄ 40:60**Flow rate:** 0.8**Injection volume:** 50**Detector:** UV 200

CHROMATOGRAM**Retention time:** 19.01 (estrone sulfate), 16.54 (equilin sulfate), 17.87 (17α-dihydroequilin sulfate), 16.16 (17β-dihydroequilin sulfate), 25.28 (17α-estradiol sulfate), 19.01 (17β-estradiol sulfate), 13.88 (equilenin sulfate), 14.26 (17α-dihydroequilenin sulfate)

KEY WORDS

injections; tablets

REFERENCEFlann, B.; Lodge, B. Analysis of estrogen sulphate mixtures in pharmaceutical formulations by reversed-phase chromatography. *J.Chromatogr.*, **1987**, *402*, 273-282

SAMPLE**Matrix:** formulations**Sample preparation:** Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Ultrasphere Octyl**Mobile phase:** MeOH:water:trichloroethanol 23:75:2, all 0.1 M in silver nitrate**Column temperature:** 45**Flow rate:** 1.0

Injection volume: 10

Detector: UV 270

CHROMATOGRAM

Retention time: 50.61 (estrone sulfate), 33.40 (equilin sulfate), 19.74 (17 α -dihydroequilin sulfate), 14.17 (17 β -dihydroequilin sulfate), 47.07 (17 α -estradiol sulfate), 33.43 (17 β -estradiol sulfate), 30.37 (equilenin sulfate), 23.28 (17 α -dihydroequilenin sulfate)

KEY WORDS

tablets; injections

REFERENCE

Flann, B.; Lodge, B. Analysis of estrogen sulphate mixtures in pharmaceutical formulations by reversed-phase chromatography. *J.Chromatogr.*, **1987**, *402*, 273–282

SAMPLE

Matrix: formulations

Sample preparation: Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere Octyl

Mobile phase: MeCN:MeOH:buffer 32:18:50 (Buffer was 1.7 mM cetyltrimethylammonium phosphate and 25 mM KH₂PO₄.)

Flow rate: 0.9

Injection volume: 50

Detector: UV 200

CHROMATOGRAM

Retention time: 58.33 (estrone sulfate), 53.08 (equilin sulfate), 45.50 (17 α -dihydroequilin sulfate), 34.41 (17 β -dihydroequilin sulfate), 50.75 (17 α -estradiol sulfate), 39.66 (17 β -estradiol sulfate), 48.41 (equilenin sulfate), 37.91 (17 α -dihydroequilenin sulfate), 17.50 (estrone), 15.75 (equilin), 14.00 (17 α -dihydroequilin), 11.08 (17 β -dihydroequilin), 15.75 (17 α -estradiol), 13.42 (17 β -estradiol), 14.58 (equilenin sulfate), 9.92 (17 β -dihydroequilenin)

KEY WORDS

tablets; injections

REFERENCE

Flann, B.; Lodge, B. Analysis of estrogen sulphate mixtures in pharmaceutical formulations by reversed-phase chromatography. *J.Chromatogr.*, **1987**, *402*, 273–282

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, weigh out amount corresponding to 6.9 mg conjugated estrogens, add 6 g Celite 545, add 4 mL water, mix, add to a mixture of 2 g Celite and 1 mL water in a 150 \times 25 tube, dry rinse container with 1 g Celite and add this to the tube, elute with 100 mL water-saturated ether, collect this eluate (A), elute with 5 mL 20 mg/mL dicyclohexylamine acetate in chloroform, elute with 145 mL chloroform (B). Combine the chloroform eluates and evaporate them to dryness under a stream of air on a steam bath, reconstitute with 20 mL MeOH, add 6 mL 5% HCl, reflux for 12 min, cool in an ice bath, add 5 mL 400 μ g/mL ethinyl estradiol in MeOH, add 70 mL water, add 50 mL benzene (Caution! Benzene is a carcinogen!), shake for 1 min. Remove the organic layer and wash it with 10 mL water, three 15 mL portions of 2% sodium carbonate solution, and two 10 mL portions of water. Pass the organic layer through 30 g anhydrous sodium sulfate in a column to give C. Evaporate a 2 mL aliquot of the solution (or an aliquot of eluate A) to dryness under a stream of air, reconstitute with 10 mL 200 μ g/mL

dansyl chloride in acetone, add 15 mL buffer, let stand in the dark for 30 min, add 50 mL water, add 50 mL ether, shake for several min, extract the aqueous layer with 25 mL ether. Combine the ether layers and wash them with two 25 mL portions of water, pass the organic layer through a 150×25 column containing 50 g anhydrous sodium sulfate, wash the column with 25 mL ether. Combine the eluates and evaporate them to dryness under a stream of air on a steam bath, reconstitute with 10 mL chloroform, inject an aliquot. (Under these conditions estrone, equilin, and equilenin co-elute. They can be reduced to β -estradiol, β -dihydroequilin, and β -dihydroequilenin, respectively, as follows. Evaporate a 10 mL aliquot of eluate (A) or solution (C) to dryness under a stream of air, reconstitute with 20 mL MeOH, add 150 mg sodium borohydride (Caution! Flammable hydrogen gas is evolved!), let stand for 45 min, add 70 mL water, add 50 mL benzene, shake for 1 min. Remove the organic layer and wash it with four 20 mL portions of water, pass through 30 g of anhydrous sodium sulfate in a 150×25 tube, evaporate a 10 mL aliquot to dryness and proceed with the derivatization as described above. (Prepare the buffer by dissolving 366.7 mg anhydrous sodium carbonate in 300 mL water and adding 150 mL acetone. Note that the initial elution with ether (A) gives free estrogens and the elution with chloroform (B) gives 3-sulfate derivatives which are then hydrolyzed.)

HPLC VARIABLES

Column: 250×4.6 5 μm Zorbax-Sil

Mobile phase: n-Heptane:chloroform:EtOH 50:49.5:0.5

Flow rate: 2

Injection volume: 10

Detector: F ex 240-420 (filter) em 440 (cutoff filter)

CHROMATOGRAM

Retention time: 4 (estrone), 4 (equilin), 4 (equilenin), 12.5 (α -estradiol), 15 (α -dihydroequilin), 16 (α -dihydroequilenin), 18 (β -estradiol), 19.5 (β -dihydroequilin), 22 (β -dihydroequilenin)

Internal standard: ethinyl estradiol (9)

KEY WORDS

normal phase; tablets; derivatization

REFERENCE

Roos, R.W.; Lau-Cam, C.A. Liquid chromatographic analysis of conjugated and esterified estrogens in tablets. *J.Pharm.Sci.*, **1985**, *74*, 201-204

SAMPLE

Matrix: formulations

Sample preparation: Finely powder tablets. Weigh out an amount equivalent to 3 mg piperazine estrone sulfate, add 10 mL mobile phase containing 100 $\mu\text{g}/\text{mL}$ biphenyl, shake 30 min, inject 10 μL aliquot.

HPLC VARIABLES

Column: 250×4.8 Brownlee RP-18

Mobile phase: MeCN:20 mM pH 5.0 phosphate buffer 55:45 containing 3 mM cetyltrimethylammonium bromide

Flow rate: 2

Injection volume: 10

Detector: UV 225

CHROMATOGRAM

Retention time: k' 8.06 (estrone sulfate), k' 7.63 (equilin sulfate), k' 6.45 (α -estradiol sulfate), k' 5.30 (β -estradiol sulfate), k' 2.78 (estrone), k' 2.16 (α -estradiol), k' 1.95 (β -estradiol)

Internal standard: biphenyl (k' 11.04)

Limit of detection: 1 $\mu\text{g}/\text{mL}$

OTHER SUBSTANCES

Simultaneous: methylparaben, propylparaben

KEY WORDS

tablets

REFERENCE

Carignan, G.; Lodge, B.A.; Skakum, W. Analysis of piperazine estrone sulfate in tablets by ion-pair high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *234*, 240–243

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets (60 mesh), take powder equivalent to about 3.2 mg conjugated estrogens, add 50 mL MeOH, shake 30 min, dilute to 100 mL with MeOH, mix, filter, discard first 20 mL filtrate, collect the rest of the filtrate (A). Take a 25 mL aliquot, add 1 mL HCl, add boiling chips, heat on a steam bath for 5 min, cool, add 70 mL water, extract with 75 mL benzene (Caution! Benzene is a carcinogen!). Wash the benzene layer with 15 mL water, four times with 15 mL 2% sodium carbonate in water, and twice with 10 mL water. Pass the benzene through a tube containing 30 g anhydrous sodium sulfate, wash the tube with 25 mL benzene, evaporate to dryness. Add 10 mL 200 µg/mL dansyl chloride in acetone, swirl to dissolve, add 15 mL base solution, mix, stopper, allow to stand in the dark for 30 min. Extract twice with 50 mL ether, wash each extract twice with 25 mL water, pass the ether through a 150 × 25 mm tube containing 50 g anhydrous sodium sulfate, wash the column with 25 mL ether, evaporate the ether layers to dryness, dissolve residue in 5 mL chloroform, inject a 10 µL aliquot. (Prepare base solution by dissolving 366.7 mg anhydrous sodium carbonate in 300 mL water and adding 150 mL acetone. Estrone, equilin, and equilenin co-elute. They can be reduced to β-estradiol, β-dihydroequilin, and β-dihydroequilenin, respectively as follows. Add 150 mg sodium borohydride to 25 mL filtrate (A) (Caution! Flammable hydrogen gas is evolved!), let stand for 45 min, add 1 mL HCl, let stand for 15 min, add 25 mL water, add 25 mL benzene, shake, wash the organic layer with four 20 mL portions of water, pass the organic layer through 25 g anhydrous sodium sulfate in a 150 × 25 tube. Evaporate a 1 mL aliquot to dryness under a stream of air and proceed with the derivatization as described above.)

HPLC VARIABLES

Column: 250 × 3.2 5 µm LiChrosorb Si-60

Mobile phase: n-Heptane:chloroform 50:50

Flow rate: 0.98

Injection volume: 10

Detector: F ex 240-420 em 440 (cut-off)

CHROMATOGRAM

Retention time: 5 (estrone), 5 (equilin), 5 (equilenin), 14 (α-estradiol), 16 (α-dihydroequilin), 18 (α-dihydroequilenin), 20 (β-estradiol), 21 (β-dihydroequilin), 24 (β-dihydroequilenin)

KEY WORDS

tablets; normal phase; derivatization

REFERENCE

Roos, R.W.; Medwick, T. Application of dansyl derivatization to the high pressure liquid chromatographic identification of equine estrogens. *J.Chromatogr.Sci.*, **1980**, *18*, 626–630

SAMPLE

Matrix: solutions

HPLC VARIABLES**Column:** 120 × 4.5 μm ODS-2 (Knauer)**Mobile phase:** MeCN:water 30:70 containing 16 mM β-cyclodextrin**Column temperature:** 40**Flow rate:** 1**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 3 (17β-estradiol), 5 (17α-estradiol), 6 (equilin)

REFERENCE

Lamparczyk, H.; Zarzycki, P.K. Effect of temperature on separation of estradiol stereoisomers and equilin by liquid chromatography using mobile phases modified with β-cyclodextrin. *J.Pharm. Biomed.Anal.*, **1995**, *13*, 543–549

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 2 μL aliquot of a 1 mg/mL solution in MeOH.

HPLC VARIABLES**Column:** 150 × 4.6 μm Zorbax ODS**Mobile phase:** MeOH:50 mM KH₂PO₄ 45:55 containing 5 mg/mL heptakis(2,6-di-O-methyl)-β-cyclodextrin**Injection volume:** 2**Detector:** UV 200

CHROMATOGRAM**Retention time:** 14 (equilin), 17 (estrone)

OTHER SUBSTANCES**Simultaneous:** 2-hydroxyestrone, 4-hydroxyestrone, 16α-hydroxyestrone

REFERENCE

Spencer, B.J.; Purdy, W.C. High-performance liquid chromatographic separation of equilin, estrone, and estrone derivatives with cyclodextrins as mobile phase additives. *J.Liq.Chromatogr.*, **1995**, *18*, 4063–4080

SAMPLE**Matrix:** solutions**Sample preparation:** Inject an aliquot of a solution in EtOH.

HPLC VARIABLES**Column:** 150 × 4.6 μm Spherisorb S5-ODS**Mobile phase:** Gradient. MeOH:20 mM ammonium sulfate from 30:70 to 100:0 over 35 min.**Column temperature:** 45**Flow rate:** 1**Injection volume:** 50**Detector:** F ex 214 em 340 (cut-off); UV 280

CHROMATOGRAM**Retention time:** 5 (estriol-3-sulfate), 12 (estrone-3-sulfate), 13 (17β-estradiol-3-sulfate), 16 (estriol), 22 (estrone), 23 (17β-estradiol)

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. *Bio-med.Chromatogr.*, **1990**, *4*, 34–38

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 250 × 4.6 7-8 μm Zorbax BP-ODS

Mobile phase: MeCN:water 35:65

Flow rate: 2

Injection volume: 50

Detector: UV 280

CHROMATOGRAM

Retention time: 32 (estrone), 28.5 (equilin), 25.5 (equilenin), 23 (17β-estradiol), 18.5 (17α-dihydroequilin), 16 (17α-dihydroequilenin)

KEY WORDS

also details of normal phase procedure

REFERENCE

Lin, J.-T.; Heftmann, E. High-performance liquid chromatography of naturally occurring estrogens. *J.Chromatogr.*, **1981**, *212*, 239–244

SAMPLE

Matrix: urine

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL water, 5 mL MeOH, and 10 mL water. 1 mL Urine + 2 nmoles equilin + 100 μL 1.5 M pH 3 acetate buffer, add to the SPE cartridge, wash with 10 mL 150 mM pH 3 acetate buffer, elute with 3 mL MeOH. Add HCl to the eluate so that the concentration of HCl is 500 mM, heat at 100° for 1.5 h, neutralize with sodium bicarbonate, extract with 2 mL chloroform. Evaporate the organic layer to dryness, reconstitute with 1 mL 5 μM 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) in MeCN containing 25 nM 18-crown-6 and 15 mM potassium carbonate, heat at 80° for 30 min, filter, inject a 10-15 μL aliquot.

HPLC VARIABLES

Column: 5 μm Hypersil ODS

Mobile phase: MeOH:water 75:25

Flow rate: 1

Injection volume: 10-15

Detector: UV 380

CHROMATOGRAM

Retention time: 6.50 (estrone), 8.39 (estradiol), 3.23 (estriol)

Internal standard: equilin (4.95)

Limit of detection: 30-50 nM

KEY WORDS

derivatization; SPE; derivatives are not fluorescent

REFERENCE

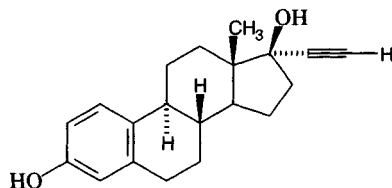
Tirendi, S.; Lancetta, T.; Bousquet, E. Estrogens determination in urine by RP-HPLC with UV detection. *Farmaco*, **1994**, *49*, 427–430

Ethinyl Estradiol

Molecular formula: C₂₀H₂₄O₂

Molecular weight: 296.4

CAS Registry No.: 57-63-6



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 500 μ L 10 M NaOH, shake on a slow rotatory mixer for 5 min, add 5 mL diethyl ether, rotomix 10 min, centrifuge at 700 g for 5 min, repeat extraction. Combine organic layers, evaporate to dryness under a stream of nitrogen at 37°, dissolve in 250 μ L mobile phase, inject aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 μ m Novapack C18

Mobile phase: MeCN:MeOH:buffer 35:15:50 (Buffer was 50 mM KH₂PO₄ adjusted to pH 3.6 with phosphoric acid.)

Flow rate: 1.6

Injection volume: 50

Detector: E, Waters Model 464 pulsed electrochemical detector, + 1 V versus Ag/AgCl

CHROMATOGRAM

Retention time: 2.94

Limit of detection: 50 pg/mL

OTHER SUBSTANCES

Simultaneous: estradiol, estriol, estrone, heparin

Noninterfering: pentobarbital

KEY WORDS

plasma; rabbit

REFERENCE

Fernández, N.; Garcia, J.J.; Diez, M.J.; Terán, M.T.; Sierra, M. Rapid high-performance liquid chromatographic assay of ethinyloestradiol in rabbit plasma. *J.Chromatogr.*, **1993**, 619, 143–147

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 10 μ L 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 μ L MeCN. Add 10 μ L reagent 1, add 10 μ L reagent 2, heat at 50° for 15 min, cool to room temperature, add 100 μ L water, add 200 μ L 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 μ L by evaporation at 40° under reduced pressure, inject 20 μ L 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.)

HPLC VARIABLES

Guard column: 50 \times 4.5 μ m Wakosil 5C18

Column: 300 \times 4.5 μ m Wakosil 5C18

Mobile phase: MeOH:water 90:10

Flow rate: 0.7
Injection volume: 20
Detector: F ex 336 em 440

CHROMATOGRAM

Retention time: 15.4
Internal standard: sec-butyl p-hydroxybenzoate (14.3)
Limit of detection: 1-2 pg/mL

OTHER SUBSTANCES

Simultaneous: equilenin, equilin, estetrol, estradiol, estriol, estrone, 2-hydroxyestradiol, 4-hydroxyestradiol

KEY WORDS

plasma; equilin and equilenin not resolved

REFERENCE

Katayama, M.; Taniguchi, H. Determination of estrogens in plasma by high-performance liquid chromatography after pre-column derivatization with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole. *J.Chromatogr.*, **1993**, *616*, 317-322

SAMPLE

Matrix: blood, perfusate
Sample preparation: 200 μ L Plasma or perfusate + 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 μ L mobile phase, inject a 50 μ L 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 (17 α -ethynylestradiol)

OTHER SUBSTANCES

Extracted: digoxin

KEY WORDS

plasma; rat; ethinyl estradiol is IS

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: bulk

HPLC VARIABLES

Column: 250 \times 4 10 μ m LiChrosorb RP-18
Mobile phase: MeOH:water 70:30
Flow rate: 1

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: impurities, norethindrone

Interfering: norgestrel

REFERENCE

Görög, S.; Herényi, B. Analysis of steroids. XXXVIII. The use of high-performance liquid chromatography with diode-array UV detection for estimating impurity profiles of steroid drugs. *J.Chromatogr.*, **1987**, *400*, 177-186

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 12 tablets in 600 mL water with stirring at 75 rpm, remove 3 mL sample, centrifuge at 3000 rpm for 15 min, inject a 250 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Phenomenex IB-Sil 3 C18

Mobile phase: MeCN:water 40:60, pH 5.6

Flow rate: 1.2

Injection volume: 250

Detector: UV 200

CHROMATOGRAM

Retention time: 9.5

OTHER SUBSTANCES

Simultaneous: norethindrone

KEY WORDS

tablets; modification of USP method

REFERENCE

Dorantes, A.; Stavchansky, S. Modification of the U.S.P. dissolution method for the analysis of norethindrone and ethinyl estradiol tablets. *J.Pharm.Sci.*, **1994**, *83*, 379-381

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 6 tablets in 600 mL dissolution medium (100 mM HCl + 0.02% sodium lauryl sulfate), remove 5 mL samples, centrifuge at 1500 rpm for 10 min, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Column: 220 \times 4.6 5 μ m Spheri-5 C18

Mobile phase: MeCN:20 mM pH 6.0 phosphate buffer 35:65

Flow rate: 1.5

Injection volume: 50-200

Detector: UV 200

CHROMATOGRAM

Retention time: 22.73

OTHER SUBSTANCES**Simultaneous:** norethindrone

KEY WORDS

tablets; modified USP method

REFERENCE

Nguyen, H.T.; Shiu, G.K.; Worsley, W.N.; Skelly, J.P. Dissolution testing of norethindrone:ethinyl estradiol, norethindrone:mestranol, and norethindrone acetate:ethinyl estradiol combination tablets. *J.Pharm.Sci.*, **1990**, *79*, 163–167

SAMPLE**Matrix:** formulations**Sample preparation:** 5 Tablets + 2 glass beads + 25 mL 50 µg/mL dibutyl phthalate in MeOH, vortex 15 min or until tablets have completely disintegrated, sonicate 5 min, filter (2 µm), inject 25 µL aliquot.

HPLC VARIABLES**Column:** 50 × 4.5 5µm IBM C18**Mobile phase:** MeOH:THF:water 10:25:65**Flow rate:** 2.1**Injection volume:** 25**Detector:** UV 230

CHROMATOGRAM**Retention time:** 3.5**Internal standard:** dibutyl phthalate

OTHER SUBSTANCES**Simultaneous:** degradation products, norgestimate

KEY WORDS

tablets; stability-indicating

REFERENCE

Lane, P.A.; Mayberry, D.O.; Young, R.W. Determination of norgestimate and ethinyl estradiol in tablets by high-performance liquid chromatography. *J.Pharm.Sci.*, **1987**, *76*, 44–47

SAMPLE**Matrix:** formulations**Sample preparation:** Powder tablets (60 mesh), weigh out amount equivalent to one tablet, add 2 mL 50 µg/mL BHT in MeCN:water 80:20, shake 30 min, centrifuge.

HPLC VARIABLES**Column:** 250 × 3.2 Altex RP-2 express series**Mobile phase:** MeCN:water 38:62**Flow rate:** 1.75**Injection volume:** 20**Detector:** UV 210; UV 280

CHROMATOGRAM**Retention time:** k' 3.85**Internal standard:** BHT (butylated hydroxytoluene) (k' 16.54)

OTHER SUBSTANCES

Simultaneous: degradation products, ethynodiol diacetate, mestranol

KEY WORDS

tablets

REFERENCE

Carignan, G.; Lodge, B.A.; Skakum, W. Quantitative analysis of ethynodiol diacetate and ethinyl estradiol/mestranol in oral contraceptive tablets by high-performance liquid chromatography. *J.Pharm.Sci.*, **1982**, *71*, 264-266

SAMPLE

Matrix: formulations

Sample preparation: 1 Tablet + 4 mL 50 mM KH_2PO_4 , rotate 15 min, add 2 mL 1 $\mu\text{g}/\text{mL}$ o-phenylphenol in mobile phase, add 4 mL MeOH, rotate 15 min, centrifuge. Remove supernatant, extract residue twice with 5 mL mobile phase (10 min rotation), combine supernatants, inject 50 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 10 μm LiChrosorb RP8

Mobile phase: MeOH:50 mM KH_2PO_4 3:2

Flow rate: 2

Injection volume: 50

Detector: F ex 280 em 330

CHROMATOGRAM

Retention time: 9

Internal standard: o-phenylphenol (6)

OTHER SUBSTANCES

Interfering: norethindrone

KEY WORDS

tablets; stability-indicating

REFERENCE

Strusiak, S.H.; Hoogerheide, J.G.; Gardner, M.S. Determination of ethinyl estradiol in solid dosage forms by high-performance liquid chromatography. *J.Pharm.Sci.*, **1982**, *71*, 636-640

SAMPLE

Matrix: media

Sample preparation: Extract culture medium twice with 2 volumes of ether, combine the extracts and evaporate them to dryness, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 Techopak 10 C18 (HPLC Technology)

Mobile phase: MeOH:0.5% pH 3.0 $(\text{NH}_4)\text{H}_2\text{PO}_4$ 62:38

Flow rate: 0.7

Detector: UV 280; Radioactivity

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES**Extracted:** estrone**Interfering:** estradiol

KEY WORDS

culture medium; tritium labeled

REFERENCE

Wild, M.J.; Rudland, P.S.; Back, D.J. Metabolism of the oral contraceptive steroids ethynylestradiol and norgestimate by normal (Huma 7) and malignant (MCF-7 and ZR-75-1) human breast cells in culture. *J.Steroid Biochem.Mol.Biol.*, **1991**, 39, 535-543

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 50 × 4.6 5 μm Supelcosil LC-18**Mobile phase:** MeOH:THF:water 10:20:70**Flow rate:** 2**Injection volume:** 20**Detector:** UV 220

CHROMATOGRAM**Retention time:** 8.8

OTHER SUBSTANCES**Simultaneous:** norethindrone, norethindrone acetate, norethynodrel acetate, norgestrel

REFERENCE*Supelco Catalog*, **1994**, p. 779

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 280

CHROMATOGRAM**Retention time:** k' 3.204

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone (UV 240), medroxyprogesterone acetate (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone (UV 240), methylprednisolone acetate (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone (UV 240), prednisolone acetate (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

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C18

Mobile phase: MeCN:THF:water 12.9:22.4:64.7

Flow rate: 1

Detector: UV 240

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES

Simultaneous: estrone, mestranol, norethindrone, norethindrone acetate, norgestrel

REFERENCE

Gazdag, M.; Szepesi, G.; Szelezcki, E. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. I. Optimization for selectivity in reversed-phase chromatography. *J.Chromatogr.*, **1988**, *454*, 83–94

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb Si 60

Mobile phase: Hexane:dioxane:isopropanol 95:3:2 (Caution! Dioxane is a carcinogen!)

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 17

OTHER SUBSTANCES

Simultaneous: estrone, mestranol, norethindrone, norethindrone acetate, norgestrel

KEY WORDS

normal phase

REFERENCE

Gazdag, M.; Szepesi, G.; Fábíán-Varga, K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. II. Optimization for selectivity in normal-phase systems. *J.Chromatogr.*, **1988**, *454*, 95–107

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 200 × 4 5 μm Nucleosil RP-8

Mobile phase: MeCN:MeOH:water 3:76:21

Flow rate: 1

Injection volume: 20

Detector: UV 205

CHROMATOGRAM

Retention time: 2.8

OTHER SUBSTANCES

Simultaneous: metabolites, desogestrel, 3-hydroxydesogestrel, 3-ketodesogestrel, 6-ketothinyl estradiol

REFERENCE

Smilde, A.K.; Bruins, C.H.P.; Doornbos, D.A.; Vink, J. Optimization of the reversed-phase high-performance liquid chromatographic separation of synthetic estrogenic and progestogenic steroids using the multi-criteria decision making method. *J.Chromatogr.*, **1987**, *410*, 1-12

SAMPLE

Matrix: solutions

Sample preparation: Extract 15 mL water with dichloromethane, evaporate organic layer, take up residue in 3 mL mobile phase, inject 50 μ L aliquot.

HPLC VARIABLES

Column: reverse phase

Mobile phase: MeOH:water 82:18

Injection volume: 50

Detector: F ex 200 em 300

CHROMATOGRAM

Internal standard: mestranol

Limit of quantitation: 10 ng/mL

REFERENCE

de Leede, L.G.J.; Govers, C.P.M.; de Nijs, H. A multi-compartment vaginal ring system for independently adjustable release of contraceptive steroids. *Contraception*, **1986**, *34*, 589-602

SAMPLE

Matrix: solutions

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

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

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

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 1.45

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: tissue

Sample preparation: Incubate endometrial tissue with buffer, remove tissue, extract medium twice with 2 volumes of diethyl ether, evaporate to dryness, reconstitute in a small volume of MeOH, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Technopak 10 C18

Mobile phase: MeOH:0.5% pH 3.0 (NH₄)H₂PO₄ 62:38

Flow rate: 0.7

Detector: UV 280

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

endometrial tissue

REFERENCE

Wild, M.J.; Rudland, P.S.; Back, D.J. Metabolism of the oral contraceptive steroids ethynylestradiol, norgestimate and 3-ketodesogestrel by a human endometrial cancer cell line (HEC-1A) and endometrial tissue *in vitro*. *J.Steroid Biochem.Mol.Biol.*, **1993**, *45*, 407–420

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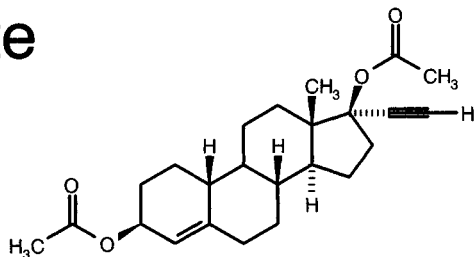
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- Standeven, A.M.; Shi, Y.E.; Sinclair, J.F.; Sinclair, P.R.; Yager, J.D. Metabolism of the liver tumor promoter ethinyl estradiol by primary cultures of rat hepatocytes. *Toxicol.Appl.Pharmacol.*, **1990**, *102*, 486–496 [gradient; UV detection; radioactivity detection; extracted metabolites]
- Backe, W. [Determination of ethinyl estradiol in feces of calves and cattle with high pressure liquid chromatography]. *Arch.Pharm.(Weinheim)*, **1988**, *321*, 431–432
- Lee, G.J.-L.; Oyang, M.-H.; Bautista, J.; Kushinsky, S. Determination of ethynylestradiol and norethindrone in a single specimen of plasma by automated high-performance liquid chromatography and subsequent radioimmunoassay. *J.Liq.Chromatogr.*, **1987**, *10*, 2305–2318 [extracted norethindrone; plasma; RIA detection; LOD 20 pg/mL]
- Nielen, M.W.F.; van Soest, R.E.J.; van Ingen, H.E.; Farjam, A.; Frei, R.W.; Brinkman, U.A.T. Selective on-line trace enrichment for the determination of ethinyl steroids in urine by liquid chromatography with precolumn technology. *J.Chromatogr.*, **1987**, *417*, 159–167 [column-switching; urine]
- Reif, V.D.; Eickhoff, W.M.; Jackman, J.K.; DeAngelis, N.J. Automated stability-indicating high-performance liquid chromatographic assay for ethinyl estradiol and (levo)norgestrel tablets. *Pharm.Res.*, **1987**, *4*, 54–58
- Roos, R.W.; Lau-Cam, C.A. Liquid chromatographic analysis of conjugated and esterified estrogens in tablets. *J.Pharm.Sci.*, **1985**, *74*, 201–204 [ethinyl estradiol is IS; derivatization; fluorescence detection]
- Swynnerton, N.F.; Fischer, J.B. Determination of ethynylestradiol and norethindrone in synthetic intestinal fluid and in timed-release oral formulations. *J.Liq.Chromatogr.*, **1980**, *3*, 1195–1204

Ethinodiol Diacetate

Molecular formula: C₂₄H₃₂O₄

Molecular weight: 384.5

CAS Registry No.: 297-76-7 (ethinodiol diacetate),
1231-93-2 (ethinodiol)



SAMPLE

Matrix: formulations

Sample preparation: Powder tablets (60 mesh), weigh out amount equivalent to one tablet, add 2 mL 50 µg/mL BHT in MeCN:water 80:20, shake 30 min, centrifuge

HPLC VARIABLES

Column: 250 × 3.2 Altex RP-2 express series

Mobile phase: MeCN:water 38:62

Flow rate: 1.75

Injection volume: 20

Detector: UV 210; UV 280

CHROMATOGRAM

Retention time: k' 22.65

Internal standard: BHT (butylated hydroxytoluene) (k' 16.54)

OTHER SUBSTANCES

Simultaneous: degradation products, ethinyl estradiol, mestranol

KEY WORDS

tablets

REFERENCE

Carignan, G.; Lodge, B.A.; Skakum, W. Quantitative analysis of ethinodiol diacetate and ethinyl estradiol/mestranol in oral contraceptive tablets by high-performance liquid chromatography. *J.Pharm.Sci.*, **1982**, *71*, 264-266

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 µL aliquot of a 30 µg/mL solution.

HPLC VARIABLES

Column: 50 × 4.6 5 µm Supelcosil LC-DP

Mobile phase: MeCN:THF:water 26:14:60

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol, mestranol, norethindrone, norethindrone acetate, norethynodrel acetate, norgestrel

REFERENCE

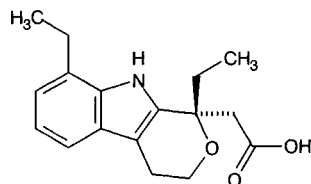
Supelco Chromatography Products, 1996, p. A130

Etodolac

Molecular formula: C₁₇H₂₁NO₃

Molecular weight: 287.4

CAS Registry No.: 41340-25-4



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)) KH₂PO₄ 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: 9.13

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glibipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, naltrexone, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide,

pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thiopropazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: amitriptyline, fluoxetine, maprotiline, nortriptyline, tropatenine

KEY WORDS

whole blood; plasma

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 or plasma + 4 mL 1 M HCl + 5 mL hexane:isopentyl alcohol 95:5, shake mechanically for 15 min, centrifuge at 1000 rpm for 5 min. Remove a 4 mL aliquot of the organic layer and add it to 1 mL 100 mM pH 11.0 Tris buffer, shake mechanically for 15 min. Remove an 800 μ L aliquot of the aqueous layer and add it to 20 μ L 2.5 M phosphoric acid, inject a 50-150 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb ODS

Mobile phase: MeCN:100 mM pH 6.0 potassium phosphate 30:70

Column temperature: 50

Flow rate: 1.8

Injection volume: 50-150

Detector: UV 226

CHROMATOGRAM

Retention time: 5.0

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, indomethacin, salicylic acid

Noninterfering: dicumarol, phenylbutazone, ethacrynic acid, glyburide, hydrochlorothiazide, niacin, phenobarbital, propoxyphene, diazepam

KEY WORDS

rat; dog; plasma; human; serum

REFERENCE

Cosyns, L.; Spain, M.; Kraml, M. Sensitive high-performance liquid chromatographic method for the determination of etodolac in serum. *J. Pharm. Sci.*, **1983**, *72*, 275-277

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 50 μ L 100 μ g/mL IS in 10 mM NaOH + 100 μ L 0.6 M sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 2500 rpm for 5 min. Remove organic layer and evaporate it to dryness. Reconstitute in 200 μ L MeCN:water 1:1, inject aliquot

HPLC VARIABLES**Column:** 100 × 4.6 Partisil 5 ODS-3**Mobile phase:** MeCN:triethylamine:70 mM KH₂PO₄ 35:0.02:65**Flow rate:** 1**Injection volume:** 5-50**Detector:** UV 280

CHROMATOGRAM**Retention time:** 6.8**Internal standard:** (±)-2-(4-benzoylphenyl)butyric acid

KEY WORDSplasma

REFERENCE

Jamali, F.; Mehvar, R.; Lemko, C.; Eradiri, O. Application of a stereospecific high-performance liquid chromatography assay to a pharmacokinetic study of etodolac enantiomers in humans. *J.Pharm.Sci.*, 1988, 77, 963-966

SAMPLE**Matrix:** blood, urine

Sample preparation: Plasma. 500 µL Plasma + 50 µL 100 µg/mL IS in 10 mM NaOH + 100 µL 0.6 M sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 2500 rpm for 5 min. Remove organic layer and evaporate it to dryness. Reconstitute in 200 µL 50 mM triethylamine in MeCN, add 50 µL 6 mM ethyl chloroformate in MeCN, after 30 s add 50 µL 0.5 M S(-)-α-methylbenzylamine in MeCN:triethylamine 80:20, after 2 min add 500 µL 0.25 M HCl, add 3 mL chloroform, vortex 15 s, centrifuge at 2500 rpm for 2 min. Remove organic layer and evaporate it. Dissolve residue in 200 µL mobile phase, inject 5-50 µL aliquot. Urine. 500 µL Urine + 250 µL 1 M NaOH, add 300 µL sulfuric acid, proceed as for plasma (above).

HPLC VARIABLES**Guard column:** 5 cm 30-38 µm HC Pellosil**Column:** 250 × 4.6 5 µm Partisil 5**Mobile phase:** Hexane:ethyl acetate:isopropanol 85:15:0.2**Flow rate:** 2**Injection volume:** 5-50**Detector:** UV 280

CHROMATOGRAM**Retention time:** 21.9 (R), 29.5 (S)**Internal standard:** (±)-2-(4-benzoylphenyl)butyric acid (10, 17)**Limit of detection:** <100 ng/mL

KEY WORDSplasma; normal phase; chiral; derivatization

REFERENCE

Jamali, F.; Mehvar, R.; Lemko, C.; Eradiri, O. Application of a stereospecific high-performance liquid chromatography assay to a pharmacokinetic study of etodolac enantiomers in humans. *J.Pharm.Sci.*, 1988, 77, 963-966

SAMPLE**Matrix:** bulk

Sample preparation: 10 mg Etodolac + 10 mg 1-(3-dimethylaminopropyl)-3-ethylcarbo-diimide hydrochloride + 2 drops 3,5-dimethylaniline + 1.5 mL dichloromethane, mix,

after 30 min add 1 mL 1 M HCl, shake vigorously. Remove the lower organic layer and dry it over anhydrous magnesium sulfate, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm D N-(3,5-dinitrobenzoyl)phenylglycine (Regis)

Mobile phase: Hexane:isopropanol 80:20

Flow rate: 2

Injection volume: 20

Detector: UV 254; UV 280

CHROMATOGRAM

Retention time: k' 1.33 (for first enantiomer)

OTHER SUBSTANCES

Also analyzed: carprofen, cicloprofen, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, naproxen, piroprofen, tiaprofenic acid

KEY WORDS

derivatization; $\alpha = 1.35$; chiral

REFERENCE

Pirkle, W.H.; Murray, P.G. The separation of the enantiomers of a variety of non-steroidal anti-inflammatory drugs (NSAIDS) as their anilide derivatives using a chiral stationary phase. *J.Liq.Chromatogr.*, **1990**, *13*, 2123–2134

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a 500 μg/mL solution in MeOH.

HPLC VARIABLES

Column: 250 × 4.6 5 μm CSP 2 polymeric chiral stationary phase (preparation details in paper)

Mobile phase: n-Hexane:EtOH:acetic acid 99:1:0.005

Column temperature: 25

Flow rate: 2

Detector: UV 220

CHROMATOGRAM

Retention time: k' 0.33 (for first enantiomer)

OTHER SUBSTANCES

Also analyzed: carprofen, cicloprofen, fenoprofen, flurbiprofen, ibuprofen, naproxen, piroprofen

KEY WORDS

$\alpha = 1.76$; chiral

REFERENCE

Terfloth, G.J.; Pirkle, W.H.; Lynam, K.G.; Nicolas, E.C. Broadly applicable polysiloxane-based chiral stationary phase for high-performance liquid chromatography and supercritical fluid chromatography. *J.Chromatogr.A*, **1995**, *705*, 185–194

SAMPLE

Matrix: solutions

Sample preparation: Dilute 1 mL buffer solution with 4 mL mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.1 5 μ m Spherisorb ODS
Mobile phase: MeCN:50 mM KH_2PO_4 45:55
Flow rate: 2
Injection volume: 40
Detector: UV 230

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Lee, Y.J.; Padula, J.; Lee, H.K. Kinetics and mechanisms of etodolac degradation in aqueous solutions. *J.Pharm.Sci.*, **1988**, *77*, 81–86

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μ L aliquot of a 300 μ g/mL solution in hexane:isopropanol 80:20.

HPLC VARIABLES

Column: 250 \times 4.6 Bakerbond-DNBPG 07651-2-20
Mobile phase: Hexane:isopropanol 99:1
Flow rate: 1
Injection volume: 10
Detector: UV 274

KEY WORDS

chiral

REFERENCE

Demerson, C.A.; Humber, L.G.; Abraham, N.A.; Schilling, G.; Martel, R.R.; Pace-Asciak, C. Resolution of etodolac and antiinflammatory and prostaglandin synthetase inhibiting properties of the enantiomers. *J.Med.Chem.*, **1983**, *26*, 1778–1780

SAMPLE

Matrix: urine
Sample preparation: Dilute a 100-200 μ L aliquot of urine to 1.1 mL with water, add 200 μ L 1 M HCl, extract with 3 mL cyclohexane:ethyl acetate 95:5. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L 100 μ g/mL ibuprofen in MeCN, inject a 20 μ L aliquot. (Hydrolyze conjugates by adding 100 μ L 1 M NaOH and vortexing twice for 15 s periods, proceed as above.)

HPLC VARIABLES

Guard column: 30 \times 4 10 μ m LiChrospher 60 CN
Column: 250 \times 4 5 μ m LiChrospher 100 RP-18
Mobile phase: MeCN:50 mM pH 4.0 phosphate buffer 45:55
Flow rate: 1.3
Injection volume: 20
Detector: UV 220

CHROMATOGRAM**Retention time:** 12.5**Internal standard:** ibuprofen (16.9)**Limit of quantitation:** 125 ng/mL

KEY WORDS

pharmacokinetics

REFERENCE

Becker-Scharfenkamp, U.; Blaschke, G. Evaluation of the stereoselective metabolism of the chiral analgesic drug etodolac by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *621*, 199-207

SAMPLE**Matrix:** urine

Sample preparation: Dilute a 100-200 μ L aliquot of urine to 1.1 mL with water, add 200 μ L 1 M HCl, extract with 3 mL cyclohexane:ethyl acetate 95:5. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L isopropanol, inject a 20 μ L aliquot. (Hydrolyze conjugates by adding 100 μ L 1 M NaOH and vortexing twice for 15 s periods, proceed as above.)

HPLC VARIABLES**Guard column:** 30 \times 4 10 μ m LiChrosorb NH2**Column:** 125 \times 4 bovine serum albumin on silica, cross-linked with formaldehyde**Mobile phase:** Isopropanol:50 mM pH 7.0 phosphate buffer 7:93**Flow rate:** 1**Injection volume:** 20**Detector:** UV 220

CHROMATOGRAM**Retention time:** 14 (S-(+)), 22 (R-(-))**Limit of quantitation:** 125 ng/mL

KEY WORDS

pharmacokinetics; chiral

REFERENCE

Becker-Scharfenkamp, U.; Blaschke, G. Evaluation of the stereoselective metabolism of the chiral analgesic drug etodolac by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *621*, 199-207

SAMPLE**Matrix:** urine

Sample preparation: Hydrolyze 1 mL urine in 200 mM pH 4.6 sodium acetate buffer with 40000 U Glusulase (DuPont) at 37° for 20 h, extract with 20 g neutral Amberlite XAD-2 resin. Elute the resin with 40 mL MeOH and methylate with excess (trimethylsilyl)diazomethane to give N-methyl etodolac methyl ester.

HPLC VARIABLES**Column:** 250 \times 4.6 Microsorb C18**Mobile phase:** MeCN:water 75:25**Flow rate:** 1.5**Detector:** UV 226

REFERENCE

Humber, L.G.; Ferdinandi, E.; Demerson, C.A.; Ahmed, S.; Shah, U.; Mobilio, D.; Sabatucci, J.; De Lange, B.; Labbadia, F.; Hughes, P.; DeVirgilio, J.; Neuman, G.; Chau, T.T.; Weichman, B.M. Etodolac, a novel antiinflammatory agent. The syntheses and biological evaluation of its metabolites. *J.Med.Chem.*, **1988**, *31*, 1712–1719

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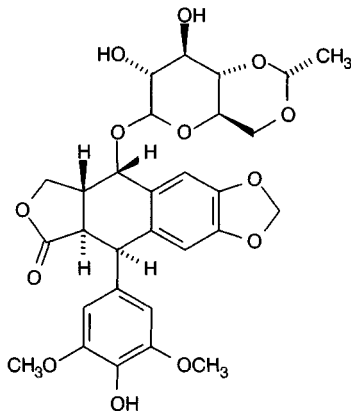
Caccamese, S. Direct high-performance liquid chromatography (HPLC) separation of etodolac enantiomers using chiral stationary phases. *Chirality*, **1993**, *5*, 164–167

Etoposide

Molecular formula: C₂₉H₃₂O₁₃

Molecular weight: 588.6

CAS Registry No.: 33419-42-0, 117091-64-2 (phosphate)



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 50 μ L 1 μ g/mL 17 β -estradiol + 200 μ L 200 mM pH 8.0 Na₂HPO₄, extract into ethylene dichloride, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L MeCN:MeOH:water 30:15:55, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 5 μ m Zorbax phenyl

Mobile phase: MeCN:MeOH:water:acetic acid 30:15:54.5:0.5, containing 10 mM tetramethylammonium hydroxide

Flow rate: 1

Injection volume: 50

Detector: E, +0.5 V

CHROMATOGRAM

Internal standard: 17 β -estradiol

Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; dog; pharmacokinetics

REFERENCE

Igwemezie, L.N.; Kaul, S.; Barbhaiya, R.H. Assessment of toxicokinetics and toxicodynamics following intravenous administration of etoposide phosphate in beagle dogs. *Pharm.Res.*, **1995**, *12*, 117-123

SAMPLE

Matrix: blood

Sample preparation: Sonicate 50 million leukemic cells in 1 mL phosphate buffered saline. 500 μ L Plasma or 1 mL sonicated cells + 0.5 (cells) or 2.5 (plasma) μ g teniposide + 2 mL chloroform, mix. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L MeOH:water 50:50, sonicate for 5 min, inject a 100 μ L aliquot. To measure non-protein-bound etoposide filter (Amicon Centrifree) while centrifuging at 20°, inject a 100-200 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb Phenyl

Mobile phase: MeOH:water:acetic acid 45:54:1

Flow rate: 1

Injection volume: 100-200

Detector: F ex 220 em 330

CHROMATOGRAM

Retention time: 6.1

Internal standard: teniposide (9.3)

Limit of detection: 10 (blood), 25 (ultrafiltrate) ng/mL

OTHER SUBSTANCES

Extracted: cis-etoposide

KEY WORDS

plasma; cells; ultrafiltrate; pharmacokinetics

REFERENCE

Liliemark, E.; Petterson, B.; Peterson, C.; Liliemark, J. High-performance liquid chromatography with fluorometric detection for monitoring of etoposide and its *cis*-isomer in plasma and leukaemic cells. *J.Chromatogr.B*, **1995**, *669*, 311-317

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Blood + 1 mL 150 ng/mL teniposide in dichloromethane: hexane 1:1, vortex for 1 min, centrifuge at 15000 g for 3 min. Remove the supernatant and evaporate it under reduced pressure at 40° for 30 min, reconstitute with 60 μ L MeCN:water:650 mM pH 4.0 sodium citrate 40:60:0.4, inject a 30 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 3.9 10 μ m μ Bondapak phenyl

Column: 150 \times 3.9 10 μ m μ Bondapak phenyl

Mobile phase: MeCN:water:650 mM pH 4.0 sodium citrate 35:57.3:7.7. When run is over wash with MeCN:water:650 mM pH 4.0 sodium citrate 70:27.3:7.7 for 4 min, re-equilibrate with initial mobile phase for 9 min.

Flow rate: 2

Injection volume: 30

Detector: E, ESA 5100A detector, Model 5020 guard cell between pump and autosampler +0.7 V, Model 5011 dual electrode analytical cell, upstream (screening) electrode +0.2 V, downstream electrode +0.45 V against Ag/AgCl.

CHROMATOGRAM

Retention time: 2.4

Internal standard: teniposide (5.8)

Limit of detection: 2.4 ng/mL

Limit of quantitation: 7.5 ng/mL

KEY WORDS

dog; rat

REFERENCE

Eisenberg, E.J.; Eickhoff, W.M. Determination of etoposide in blood by liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1993**, *621*, 110-114

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L MeOH, vortex, add 2 mL dichloroethane, shake thoroughly for 1 min, centrifuge at 3000 g for 5 min. Remove 1.5 mL of the organic

layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μ L MeOH:water 70:30, sonicate for 6 min, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 4.6 10 μ m LiChrosorb C18

Column: 100 \times 4.6 10 μ m Novapak phenyl

Mobile phase: MeOH:10 mM pH 7.0 phosphate buffer 55:45

Flow rate: 0.7

Injection volume: 10

Detector: E, Metrohm Model 641 VA, EA 286/1 glassy carbon electrode + 500 mV, stainless-steel auxiliary electrode, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.5

Internal standard: etoposide

OTHER SUBSTANCES

Extracted: teniposide

KEY WORDS

plasma; etoposide is IS

REFERENCE

van der Horst, F.A.L.; van Opstal, M.A.J.; Teeuwssen, J.; Post, M.H.; Holthuis, J.J.M.; Brinkman, U.A.T. Comparative study on the determination of the anti-neoplastic drug teniposide in plasma using micellar liquid chromatography and surfactant-mediated plasma clean-up. *J.Chromatogr.*, **1991**, 567, 161-174

SAMPLE

Matrix: blood

Sample preparation: 450 μ L Plasma + 50 μ L 380 mM sodium dodecyl sulfate in 59 mM pH 7 sodium phosphate buffer, sonicate for 5 min. Inject a 100 μ L aliquot onto column A with mobile phase A, elute with mobile phase A for 7.5 min, elute the contents of column A onto column B with mobile phase B for 1 min. After 1 min remove column A from the circuit and re-equilibrate it with mobile phase A for 1.5 min, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 \times 2.1 40 μ m Chromsep C18 (Chrompack); B 300 \times 4.6 10 μ m μ Bondapak phenyl

Mobile phase: A 10 mM pH 7.0 sodium phosphate; B MeOH:10 mM pH 7.0 sodium phosphate buffer 55:45

Flow rate: A 0.4; B 1

Injection volume: 100

Detector: UV 254; E, +500 mV vs Ag/AgCl

CHROMATOGRAM

Retention time: 4.2

Internal standard: etoposide

Limit of detection: 100 ng/mL (UV)

OTHER SUBSTANCES

Extracted: teniposide

KEY WORDS

plasma;column-switching;etoposide is IS

REFERENCE

van Opstal, M.A.J.; van der Horst, F.A.L.; Holthuis, J.J.M.; Van Bennekom, W.P.; Bult, A. Automated reversed-phase chromatographic analysis of etoposide and teniposide in plasma by using on-line surfactant-mediated sample clean-up and column-switching. *J.Chromatogr.*, **1989**, *495*, 139–151

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 3 mL chloroform, shake for 10 min, centrifuge at 1500 g for 10 min, repeat extraction. Combine the organic layers and evaporate a 5 mL aliquot to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:250 mM ammonium acetate:acetic acid 54:45:1

Flow rate: 1.5

Injection volume: 20

Detector: E, Bioanalytical Systems LC4, TL5 glassy carbon electrode, +900 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.1

Internal standard: etoposide

OTHER SUBSTANCES

Extracted: teniposide

KEY WORDS

plasma; etoposide is IS

REFERENCE

Canal, P.; Michel, C.; Bugat, R.; Soula, G.; Carton, M. Quantification of teniposide in human serum by high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1986**, *375*, 451–456

SAMPLE

Matrix: blood

Sample preparation: Mix plasma or serum with an equal volume of proteinase K, let stand for 10 min. (Alternatively, heat serum or plasma with an equal volume of 1 mg/mL subtilisin A at 50° for 15 min.) Inject 1.6 mL hydrolyzed blood or filtered serum onto column A with mobile phase A at 1 mL/min, backflush column A with mobile phase A to waste for 2 min at 2 mL/min, backflush the contents of column A onto column B with mobile phase B, after 30 s remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Clean column A by backflushing with MeOH at 2 mL/min for 3 min then forward flush with water at 1 mL/min for 2 min.)

HPLC VARIABLES

Column: A 2 × 4.6 10 µm PRP-1 divinylbenzene-styrene copolymer (Hamilton); B 125 × 4 10 µm LiChrosorb RP-18

Mobile phase: A water; B MeOH:water 55:45

Flow rate: A 1-2; B 1

Injection volume: 1600

Detector: F ex 230 em 328 following post-column extraction. The column effluent mixed with dichloroethane pumped at 0.6 mL/min, the mixture flowed through a 2 mm i.d. glass reactor (Technicon) to a phase separator (Technicon (*J.Chromatogr.* 1979, 185, 473)) with a PTFE insert and 0.3 mL/min of the organic phase flowed through the detector.

CHROMATOGRAM**Retention time:** 5**Limit of detection:** 8 ng (blood); 30 ng (urine)

OTHER SUBSTANCES**Extracted:** teniposide

KEY WORDS

column-switching; post-column extraction; plasma; serum

REFERENCE

Werkhoven-Goewie, C.E.; Brinkman, U.A.T.; Frei, R.W.; de Ruiter, C.; de Vries, J. Automated liquid chromatographic analysis of the anti-tumorigenic drugs etoposide (VP 16-213) and teniposide (VM 26). *J.Chromatogr.*, **1983**, *276*, 349-357

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 10 μ L 100 μ g/mL teniposide in MeOH + 5 mL chloroform, rock gently for 15 min, centrifuge. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, dissolve residue in 50 μ L MeOH, vortex, centrifuge for 5-10 min, inject a 25 μ L aliquot.

HPLC VARIABLES**Guard column:** 70 \times 2.1 30 μ m Co:Pell (Whatman)**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeOH:water 60:40**Flow rate:** 1**Injection volume:** 25**Detector:** F ex 215 em 328

CHROMATOGRAM**Retention time:** 5.5**Internal standard:** teniposide (8)**Limit of detection:** 25 ng/mL**Limit of quantitation:** 50 ng/mL

KEY WORDS

plasma

REFERENCE

Strife, R.J.; Jardine, I.; Colvin, M. Analysis of the anticancer drugs etoposide (VP 16-213) and teniposide (VM 26) by high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1981**, *224*, 168-174

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 10 μ L 1 mg/mL teniposide in MeOH + 5 mL chloroform, rock gently for 15 min, centrifuge. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, dissolve residue in 50 μ L MeOH, vortex, centrifuge for 5-10 min, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeOH:water 60:40**Flow rate:** 1-1.2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 5

Internal standard: teniposide (7.5)

Limit of quantitation: 500 ng/mL

KEY WORDS

plasma

REFERENCE

Strife, R.J.; Jardine, I.; Colvin, M. Analysis of the anticancer drugs VP 16-213 and VM 26 and their metabolites by high-performance liquid chromatography. *J.Chromatogr.*, **1980**, *182*, 211-220

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: 500 μ L Plasma, urine, or CSF + 500 μ L saturated ammonium sulfate + 4 mL ethyl acetate + 5 μ L 2.5 μ g/mL IS, vortex for 5 min, centrifuge at 3000 rpm for 15 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: 10 μ m μ Bondapak phenyl

Column: 250 \times 4.6 10 μ m μ Bondapak phenyl

Mobile phase: MeCN:water:acetic acid 25:74:1

Flow rate: 1

Injection volume: 50

Detector: UV 284; E, Bioanalytical Systems LC-4A, 0.85 V

CHROMATOGRAM

Retention time: 12

Internal standard: trans/cis-hydroxy acid of teniposide (26)

Limit of detection: 20 ng/mL

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Sinkule, J.A.; Evans, W.E. High-performance liquid chromatographic analysis of the semisynthetic epipodophyllotoxins teniposide and etoposide using electrochemical detection. *J.Pharm.Sci.*, **1984**, *73*, 164-168

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μ L 200 μ g/mL phenytoin or methylphenytoin + 5 mL chloroform, rotate for 10 min, centrifuge at 400 g for 10 min. Remove the organic layer, filter it, and evaporate it to dryness at 50°. Reconstitute in 200 μ L mobile phase, inject an aliquot. Urine. 200 μ L Urine + 1 mL pH 7.3 phosphate-buffered saline + 50 μ L 200 μ g/mL phenytoin or methylphenytoin + 5 mL chloroform, rotate for

10 min, centrifuge at 400 g for 10 min. Remove the organic layer, filter it, and evaporate it to dryness at 50°. Reconstitute in 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 5 μ m ODS Hypersil

Mobile phase: MeOH:water 51:49

Flow rate: 2

Injection volume: 50

Detector: UV 229

CHROMATOGRAM

Retention time: 2.1

Internal standard: phenytoin (2.9), methylphenytoin (4.5)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, acetaminophen, aspirin, cyclophosphamide, dextropropoxyphene, diamorphine, dihydrocodeine, doxorubicin, methotrexate, metoclopramide, morphine, phenobarbital, prednisone, procarbazine, prochlorperazine, vincristine

KEY WORDS

plasma

REFERENCE

Harvey, V.J.; Joel, S.P.; Johnston, A.; Slevin, M.L. High-performance liquid chromatography of etoposide in plasma and urine. *J.Chromatogr.*, **1985**, 339, 419–423

SAMPLE

Matrix: formulations

Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 μ m cyano

Mobile phase: MeCN:20 mM sodium acetate 26:74, pH adjusted to 4.0 with acetic acid

Flow rate: 1

Injection volume: 20

Detector: UV 290

CHROMATOGRAM

Retention time: 6.19

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: formulations

Sample preparation: Dilute 5 mL of the injection to 50 mL with mobile phase, add a 5 mL aliquot of this solution to 5 mL 40 µg/mL methyl p-aminobenzoate in MeCN and make up to 50 mL with mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak phenyl
Mobile phase: MeCN:20 mM pH 4.0 sodium acetate 26:74
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 16.3
Internal standard: methyl p-aminobenzoate (9)

OTHER SUBSTANCES

Simultaneous: impurities, degradation products, benzaldehyde, benzyl alcohol

KEY WORDS

injections; stability-indicating

REFERENCE

Floor, B.J.; Klein, A.E.; Muhammad, N.; Ross, D. Stability-indicating liquid chromatographic determination of etoposide and benzyl alcohol in injectable formulations. *J.Pharm.Sci.*, **1985**, *74*, 197–200

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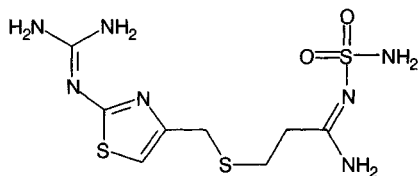
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- el-Yazigi, A.; Martin, C.R. Improved assay for etoposide in plasma by radial-compression liquid chromatography with electrochemical detection. *Clin.Chem.*, **1987**, *33*, 803–805
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- Littlewood, T.J.; Hutchings, A.L.; Bentley, D.P.; Spragg, B.P. High-performance liquid chromatographic determination of etoposide in plasma using electrochemical detection. *J.Chromatogr.*, **1984**, *336*, 434–437 [plasma; electrochemical detection; teniposide (IS); LOD 5 ng/mL]

Famotidine

Molecular formula: C₈H₁₅N₇O₂S₃

Molecular weight: 337.4

CAS Registry No.: 76824-35-6



SAMPLE

Matrix: blood

Sample preparation: Condition an SPE cartridge with 1 mL MeOH and 1 mL water. Add 1 mL plasma to the SPE cartridge, wash with 5 mL water, elute with 2 mL MeCN. Evaporate the eluate, reconstitute in acetic acid/phosphate buffer, centrifuge, inject an aliquot.

HPLC VARIABLES

Guard column: Newguard RP-8 (Applied Biosystems)

Column: Spherisorb C8

Mobile phase: MeCN:30 mM pH 2.6 NaH₂PO₄ 7:93 containing 7.2 mM triethylamine

Flow rate: 1

Detector: UV 267

CHROMATOGRAM

Retention time: 9.6

Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Schwartz, J.I.; Yeh, K.C.; Berger, M.L.; Tomasko, L.; Hoover, M.E.; Ebel, D.L.; Stauffer, L.A.; Han, R.; Bjornsson, T.D. Novel oral medication delivery system for famotidine. *J.Clin.Pharmacol.*, **1995**, *35*, 362-367

SAMPLE

Matrix: blood

Sample preparation: 1.5 mL Plasma + 100 μ L 4 M HCl, shake, add 8 mL diethyl ether, shake for 15 min, centrifuge at 1000 g at 4° for 5 min, discard ether layer. Add 0.5 mL saturated Na₂CO₃ solution, 0.5 mL saturated NaHCO₃ solution, 100 μ L 5 μ g/mL clopamide in water, and 7 mL ethyl acetate to aqueous layer, shake 15 min, centrifuge at 2000 g at 4° for 5 min, repeat extraction, combine organic layers and evaporate them to dryness under a stream of nitrogen at 37°. Reconstitute with 150 μ L MeCN:water 12:88, vortex, inject 90 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 2.5 μ m ODS Hypersil C18

Mobile phase: MeCN:water 12:88 containing 20 mM Na₂HPO₄ and 50 mM sodium dodecyl sulfate adjusted to pH 3 with orthophosphoric acid

Flow rate: 0.5

Injection volume: 90

Detector: UV 267

CHROMATOGRAM

Retention time: 13

Internal standard: clopamide (9)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, amiloride, aspirin, atenolol, chlorothiazide, cimetidine, cyclopentiazide, diazepam, furosemide, indapamide, labetalol, lorazepam, metoprolol, phenytoin, propranolol, ranitidine, salicylic acid, theophylline

KEY WORDS

plasma; microbore

REFERENCE

Wanwimolruk, S.; Zoest, A.R.; Wanwimolruk, S.Z.; Hung, C.T. Sensitive high-performance liquid chromatographic determination of famotidine in plasma. Application to pharmacokinetic study. *J.Chromatogr.*, **1991**, 572, 227-238

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL saturated potassium carbonate + 5 mL ethyl acetate, mix, centrifuge. Remove the organic layer and add it to 1 mL saturated NaCl solution and 1 mL 1 M HCl, centrifuge. Remove the aqueous phase and add it to 1 mL saturated potassium carbonate solution and 3 mL ethyl acetate, extract. Remove a 2.5 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue with 400 μ L 250 μ M phenanthrenequinone in MeOH, evaporate to dryness under reduced pressure, reconstitute with 40 μ L DMF and 40 μ L 2 M NaOH, heat at 60° for 15 min, cool on ice, neutralize with 5 M acetic acid, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: reversed phase

Mobile phase: MeCN:10 mM pH 4 citrate buffer 40:50

Flow rate: 1

Injection volume: 20

Detector: F ex 296 em 411

CHROMATOGRAM

Limit of detection: 5 ng/mL

KEY WORDS

plasma; pharmacokinetics; derivatization

REFERENCE

Echizen, H.; Shoda, R.; Umeda, N.; Ishizaki, T. Plasma famotidine concentration versus intragastric pH in patients with upper gastrointestinal bleeding and in healthy subjects. *Clin.Pharmacol.Ther.*, **1988**, 44, 690-698

SAMPLE

Matrix: blood, urine

Sample preparation: 50 μ L Plasma or urine + 350 μ L 0.2 μ g/mL 3-butylxanthine in MeOH, centrifuge at 6000 g for 5 min. Evaporate supernatant under a stream of nitrogen at 40°, reconstitute in 250 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.2 Cosmosil 5C18

Mobile phase: MeCN:30 mM pH 7.0 phosphate buffer 10:90

Flow rate: 1.5

Detector: UV 267

CHROMATOGRAM**Internal standard:** 3-butylxanthine**Limit of quantitation:** 100 ng/mL

KEY WORDS

plasma

REFERENCE

Hasegawa, T.; Nadai, M.; Wang, L.; Takayama, Y.-I.; Kato, K.; Nabeshima, T.; Kato, N. Renal excretion of famotidine and role of adenosine in renal failure induced by bacterial lipopolysaccharide in rats. *Drug Metab. Dispos.*, **1994**, *22*, 8–13

SAMPLE**Matrix:** formulations**Sample preparation:** Inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** Nova Pak C18**Mobile phase:** MeCN:0.1% acetic acid:10 mM pH 7.8 (NH₄)H₂PO₄ 10:23:74**Flow rate:** 1**Injection volume:** 20**Detector:** UV 300

CHROMATOGRAM**Retention time:** 13.9

OTHER SUBSTANCES**Simultaneous:** cefmetazole**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:** formulations**Sample preparation:** Dilute with 5% dextrose (if necessary), inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** Nova Pak C18**Mobile phase:** MeOH:100 mM (NH₄)₂HPO₄ 20:80, pH 7.80**Flow rate:** 1**Injection volume:** 20**Detector:** UV 322

CHROMATOGRAM**Retention time:** 4.6

OTHER SUBSTANCES**Simultaneous:** cefazolin

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: formulations

Sample preparation: Shake, remove 2 mL of oral suspension, dilute to 40 mL with water, vortex 1 min, centrifuge at 2000 rpm for 10 min. Dilute a 100 μ L aliquot of supernatant with 100 μ L of 100 μ g/mL theophylline and add 800 μ L water, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 300 mm 10 μ m Waters reversed-phase C18

Mobile phase: MeCN:50 mM sodium acetate buffer 8:92, adjusted to pH 6.5

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 5.6

Internal standard: theophylline (4.3)

KEY WORDS

stability-indicating

REFERENCE

Quercia, R.A.; Jay, G.T.; Fan, C.; Chow, M.S. Stability of famotidine in an extemporaneously prepared oral liquid. *Am.J.Hosp.Pharm.*, **1993**, *50*, 691-693

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m octadecylsilane (Beckman)

Mobile phase: MeOH:MeCN:glacial acetic acid:10 mM KH_2PO_4 12:3:0.1:84.9, pH 5.0

Flow rate: 1

Detector: UV 266

REFERENCE

Junnarkar, G.H.; Stavchansky, S. Isothermal and nonisothermal decomposition of famotidine in aqueous solution. *Pharm.Res.*, **1995**, *12*, 599-604

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.6 12 μ m 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' 0.54

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, 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, tripeleppamine, 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 \times 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, carbamol, 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, dapson, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyron, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, fenbendazole, fencamfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxamid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, mepredine, mepheridine, mephentermine, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, meth-

ylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, 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, tripelenamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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

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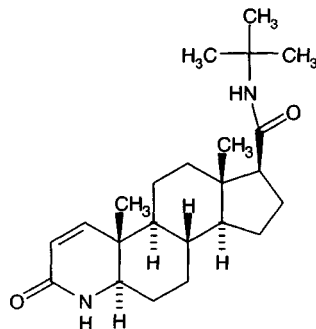
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- Kamath, B.V.; Shivram, K.; Newalkar, B.L.; Shah, A.C. Liquid chromatographic analysis and degradation kinetics of famotidine. *J.Liq.Chromatogr.*, **1993**, *16*, 1007–1014 [tablets; stability indicating]
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- Bullock, L.; Fitzgerald, J.F.; Glick, M.R. Stability of famotidine 20 and 50 mg/L in total nutrient admixtures. *Am.J.Hosp.Pharm.*, **1989**, *46*, 2326–2329 [theophylline (IS)]
- Bullock, L.; Fitzgerald, J.F.; Glick, M.R.; Parks, R.B.; Schnabel, J.G.; Hancock, B.G. Stability of famotidine 20 and 40 mg/L and amino acids in total parenteral nutrient solutions. *Am.J.Hosp.Pharm.*, **1989**, *46*, 2321–2325 [stability-indicating; theophylline (IS)]
- DiStefano, J.E.; Mitrano, F.P.; Baptista, R.J.; Der, M.M.; Silvestri, A.P.; Palombo, J.D.; Bistran, B.R. Long-term stability of famotidine 20 mg/L in a total parenteral nutrient solution. *Am.J.Hosp.Pharm.*, **1989**, *46*, 2333–2335 [non-interfering degradation products]

Finasteride

Molecular formula: C₂₃H₃₆N₂O₂

Molecular weight: 372.6

CAS Registry No.: 98319-26-7



SAMPLE

Matrix: blood

Sample preparation: 150 μ L Plasma + 150 μ L ethylene glycol:water 40:60, mix, filter (5 μ m), filter (0.22 μ m) while centrifuging at 4° at 1000 g for 5 min, inject a 150 μ L aliquot of the filtrate onto column A and elute to waste with mobile phase A, after 5 min elute column A to waste with mobile phase B, after 8 min direct the effluent from column A onto column B, after 1.5 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. Clean column A by eluting to waste with mobile phase C for 19.5 min, re-equilibrate column A with mobile phase A for 4 min.

HPLC VARIABLES

Column: A 35 \times 4.6 5 μ m Capcell Pak CN SG-120 (Shiseido); B 250 \times 4.6 5 μ m Inertsil ODS-2

Mobile phase: A MeCN:water 10:90; B MeCN:water 25:75; C MeCN:water 70:30; D MeCN:water 45:55 (Pass mobile phase A and mobile phase B through 35 \times 4.6 5 μ m Capcell Pak C18 SG-120 (Shiseido) columns before use. Clean these columns with mobile phase C each day.)

Column temperature: 40

Flow rate: C 1; D 1.1

Injection volume: 150

Detector: UV 210

CHROMATOGRAM

Retention time: 24.4

Limit of quantitation: 1 ng/mL

KEY WORDS

plasma; column-switching; heart-cut

REFERENCE

Takano, T.; Hata, S. High-performance liquid chromatographic determination of finasteride in human plasma using direct injection with column switching. *J.Chromatogr.B*, **1996**, 676, 141-146

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Baker nitrile SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma + 50 μ L 2 μ g/mL IS, vortex for 10 s, add to the SPE cartridge, wash with 2 mL acetone:water 10:90, wash with 2 mL water, elute with 250 μ L MeOH, add 10 μ L water to the eluate, inject a 200 μ L aliquot.

HPLC VARIABLES

Guard column: 25 \times 4.6 5 μ m RP-8 (Brownlee)

Column: 150 \times 4.6 5 μ m RP-8 (Altex) + 50 \times 4.6 3 μ m RP-18 (Analytichem)

Mobile phase: MeOH:MeCN:water 6:5:7

Flow rate: 1

Injection volume: 200

Detector: UV 210

CHROMATOGRAM

Retention time: 13.1

Internal standard: 4-methylfinasteride (20.8)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; SPE

REFERENCE

Constanzer, M.L.; Matuszewski, B.K.; Bayne, W.F. High-performance liquid chromatographic method for the determination of finasteride in human plasma at therapeutic doses. *J.Chromatogr.*, **1991**, 566, 127-134

SAMPLE

Matrix: blood, semen

Sample preparation: Condition a Baker 1 mL nitrile SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma or semen + 100 μ L 100 ng/mL IS, vortex for 10 s, add to the SPE cartridge, wash with 2 mL water, elute with 300 μ L MeCN, inject a 100 μ L aliquot of the eluate.

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m base deactivated C18 (Keystone)

Column: 33 \times 4.6 3 μ m C18 (Perkin-Elmer)

Mobile phase: MeCN:water 70:30 containing 0.1% formic acid

Column temperature: 70

Flow rate: 1

Injection volume: 100

Detector: MS, PE-SCIEX API III triple quadrupole, heated nebulizer, corona discharge (+5 μ A), positive ion APCI, nebulizer probe 500 $^{\circ}$, collision gas argon at 350 \times 10¹² molecules/cm², nebulizing gas nitrogen at 80 psi and 2 L/min, curtain gas nitrogen at 0.9 L/min, orifice +50 V, electron multiplier -3.8 kV, dwell time 400 ms, interface heater 60 $^{\circ}$, m/z 317

CHROMATOGRAM

Retention time: 1

Internal standard: N-(1,1,3,3-tetramethylbutyl)-3-oxo-4-aza-5 α -androst-1-ene-17 β -carboxamide (1.5)

Limit of detection: 0.2 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Constanzer, M.L.; Chavez, C.M.; Matuszewski, B.K. Picogram determination of finasteride in human plasma and semen by high-performance liquid chromatography with atmospheric-pressure chemical-ionization tandem mass spectrometry. *J.Chromatogr.B*, **1994**, 658, 281-287

SAMPLE

Matrix: microsomal incubations

Sample preparation: 500 μ L Microsomal incubation + 2 mL ice-cold water, add to a Milipore C18 SPE cartridge, elute with 5 mL MeOH. Evaporate the eluate to dryness, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 7 μ m Aquapore RP-300 (Applied Biosystems)

Column: 250 \times 4.6 5 μ m Zorbax ODS C18

Mobile phase: Gradient. A was 20 mM ammonium acetate buffer containing 0.1% trifluoroacetic acid. B was MeCN containing 0.1% trifluoroacetic acid. A:B 60:40 for 5 min, to 40:60 over 35 min.

Flow rate: 1

Detector: UV 210

CHROMATOGRAM

Retention time: 28.0

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver; SPE

REFERENCE

Huskey, S.-E.W.; Dean, D.C.; Miller, R.R.; Rasmusson, G.H.; Chiu, S.-H.L. Identification of human cytochrome P450 isozymes responsible for the in vitro oxidative metabolism of finasteride. *Drug Metab. Dispos.*, **1995**, *23*, 1126–1135

SAMPLE

Matrix: microsomal incubations

Sample preparation: Microsomal incubation + 200 μ L 5 M HCl, extract with ethyl acetate. Evaporate the ethyl acetate, take up the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: LiChrospher 100 RP-8

Column: 250 \times 4.6 Sephalyte C8 (Analytichem)

Mobile phase: MeCN:MeOH:water 35:10:55

Flow rate: 0.8-1.5

Detector: UV 210

CHROMATOGRAM

Internal standard: 17-methyltestosterone

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver

REFERENCE

Ishii, Y.; Mukoyama, H.; Ohtawa, M. In vitro biotransformation of finasteride in rat hepatic microsomes. Isolation and characterization of metabolites. *Drug Metab. Dispos.*, **1994**, *22*, 79–84

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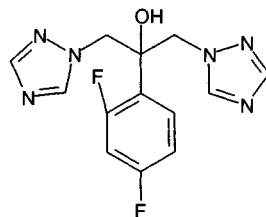
Ishii, Y.; Mukoyama, H.; Hata, S. Metabolism of finasteride in rat hepatic microsomes: age and sex differences and effects of P450 inducers. *Xenobiotica*, **1994**, *24*, 863–872 [rat; liver; microsomal incubations; extracted metabolites; 17-methyltestosterone (IS)]

Fluconazole

Molecular formula: C₁₃H₁₂F₂N₆O

Molecular weight: 306.3

CAS Registry No.: 86386-73-4



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 500 μ L 10 μ g/mL IS in MeOH:water 10:90 + 250 μ L 1 M ammonium hydroxide, mix, add 5 mL ethyl acetate, mix, centrifuge. Remove the organic layer and add it to 1 mL 1 M HCl, mix, centrifuge. Remove the aqueous layer and add it to 1.5 mL 6 M ammonium hydroxide and 5 mL ethyl acetate, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: Adsorbosphere C18

Mobile phase: MeOH:10 mM pH 7 phosphate buffer 50:50

Detector: UV 260

CHROMATOGRAM

Internal standard: UK54373

Limit of detection: 1 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Schwartz, E.L.; Hallam, S.; Gallagher, R.E.; Wiernik, P.H. Inhibition of all-*trans*-retinoic acid metabolism by fluconazole *in vitro* and in patients with acute promyelocytic leukemia. *Biochem.Pharmacol.*, 1995, 50, 923-928

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 2 column volumes MeOH and 2 column volumes 100 mM pH 6.0 sodium phosphate buffer. 1 mL Serum + 100 μ L 100 μ g/mL IS in MeOH:water 10:90 + 2 mL 100 mM pH 6.0 phosphate buffer, mix, add to the SPE cartridge, wash with 1 mL phosphate buffer, wash with 1 mL MeOH:phosphate buffer 15:85, dry under vacuum, elute with 500 μ L MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 200 μ L mobile phase, filter, inject a 50 μ L aliquot of the filtrate.

HPLC VARIABLES

Guard column: 5 μ m Adsorbosphere C18

Column: 250 \times 4.6 5 μ m Adsorbosphere C18

Mobile phase: MeCN:25 mM pH 7.0 Tris-phosphate buffer 25:75

Flow rate: 1

Injection volume: 50

Detector: UV 210

CHROMATOGRAM

Retention time: 6.6

Internal standard: 2-(4-chlorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol (UK-48,134, Pfizer) (9.0)

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: acyclovir, cefazolin, ceftazidime, clindamycin, metronidazole, piperacillin, sulfamethoxazole, trimethoprim

Noninterfering: amphotericin B

KEY WORDS

serum; SPE

REFERENCE

Inagaki, K.; Takagi, J.; Lor, E.; Okamoto, M.P.; Gill, M.A. Determination of fluconazole in human serum by solid-phase extraction and reversed-phase high-performance liquid chromatography. *Ther.Drug Monit.*, **1992**, *14*, 306–311

SAMPLE

Matrix: blood, CSF

Sample preparation: 500 μ L Serum, plasma, or CSF + 500 μ L water + 50 μ L 100 μ g/mL IS in MeOH:water 10:90 + 250 μ L 1 M ammonium hydroxide, mix, add 5 mL ethyl acetate, vortex for 30 s, centrifuge. Remove organic layer and add it to 1 mL 1 M HCl, vortex, centrifuge. Remove the aqueous layer, add it to 1.5 mL 6 M ammonium hydroxide, mix, add 5 mL ethyl acetate, mix, centrifuge. Remove the organic layer and evaporate it to dryness at 40° under a stream of nitrogen, dissolve the residue in 200 μ L mobile phase, filter (0.20 μ m), inject a 30 μ L aliquot.

HPLC VARIABLES

Guard column: Corasil C18 guard column

Column: 250 \times 4.6 5 μ m Microsorb ODS

Mobile phase: MeOH:10 mM pH 7.0 phosphate buffer 50:50

Flow rate: 1

Injection volume: 30

Detector: UV 260

CHROMATOGRAM

Retention time: 5.0

Internal standard: 2-(4-chlorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol (UK-48,134) (6.2)

Limit of quantitation: 200 ng/mL

KEY WORDS

serum; plasma; also for urine

REFERENCE

Foulds, G.; Brennan, D.R.; Wajszczuk, C.; Catanzaro, A.; Garg, D.C.; Knopf, W.; Rinaldi, M.; Weidler, D.J. Fluconazole penetration into cerebrospinal fluid in humans. *J.Clin.Pharmacol.*, **1988**, *28*, 363–366

SAMPLE

Matrix: blood, saliva

Sample preparation: Plasma. Add 100 μ L 20 μ g/mL phenacetin in MeOH to a tube, evaporate to dryness under a stream of nitrogen at 40°, add 500 μ L plasma, vortex for 10 s, add 50 μ L 5 M NaOH, vortex for 10 s, add 5 mL chloroform:isopropanol 80:20, shake for 15 min, centrifuge at 2500 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 60 μ L MeOH, vortex

for 30 s, add 140 μ L 10 mM pH 5.0 sodium acetate buffer, vortex for 30 s, centrifuge at 1000 g for 5 min, inject a 100 μ L aliquot of the supernatant. Saliva. 250 μ L Saliva + 50 μ L 5 M NaOH, vortex for 10 s, add 5 mL chloroform:isopropanol 80:20, shake for 15 min, centrifuge at 2500 g for 15 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 60 μ L MeOH, vortex for 30 s, add 140 μ L 10 mM pH 5.0 sodium acetate buffer, vortex for 30 s, centrifuge at 1000 g for 5 min, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m LiChrospher 100 RP-8

Column: 125 \times 4 5 μ m Lichrosorb RP-18

Mobile phase: MeOH:10 mM sodium acetate adjusted to pH 5.0 with concentrated HCl 30:70

Flow rate: 1

Injection volume: 100

Detector: UV 261

CHROMATOGRAM

Retention time: 8

Internal standard: phenacetin (12.5)

Limit of quantitation: 100 ng/mL (plasma), 1000 ng/mL (saliva)

OTHER SUBSTANCES

Noninterfering: acetaminophen, amphotericin B, brotizolam, ceftazidime, ciprofloxacin, codeine, diclofenac, didanosine, domperidone, fluoxetine, foscarnet, gangiclovir, methadone, metoclopramide, mianserin, nystatin, pyrimethamine, ranitidine, sulfamethoxazole, temazepam, trimethoprim, zidovudine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Koks, C.H.W.; Rosing, H.; Meenhorst, P.L.; Bult, A.; Beijnen, J.H. High-performance liquid chromatographic determination of the antifungal drug fluconazole in plasma and saliva of human immunodeficiency virus-infected patients. *J.Chromatogr.B*, **1995**, *663*, 345–351

SAMPLE

Matrix: feed

Sample preparation: Stir 5 g feed with 20 mL dichloromethane at room temperature for 1.5 h, filter, add filtrate to two 2.8 mL 500 mg Bond Elut cyanopropyl SPE cartridges in series, add the eluate to the cartridges for a second pass, dry under vacuum, elute with 20 mL MeOH:water 35:65, make up the eluate to 25 mL with MeOH:water 35:65, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 2 5 μ m Spherisorb C8

Mobile phase: MeOH:water 25:75

Column temperature: 35

Flow rate: 0.4

Detector: UV 210

KEY WORDS

SPE

REFERENCE

Khundker, S.; Dean, J.R.; Jones, P. A comparison between solid phase extraction and supercritical fluid extraction for the determination of fluconazole from animal feed. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1441-1447

SAMPLE

Matrix: injections

Sample preparation: 1 mL Sample + 50 μ L 150 ng/mL cimetidine, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.8 Spherisorb S5CN

Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH_2PO_4 adjusted to pH 5.4 with 1 M NaOH.)

Flow rate: 2.2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 2.2

Internal standard: cimetidine (3.6)

KEY WORDS

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: injections

Sample preparation: 100 μ L Injection + 700 μ L water + 100 μ L 200 μ g/mL IS in MeOH:water 10:90 + 100 μ L 1 M ammonium hydroxide, mix, add 2.5 mL ethyl acetate, vortex for 1 min, centrifuge. Remove organic layer and add it to 1 mL 1 M HCl, mix, centrifuge. Remove the aqueous layer, add 1 mL 6 M ammonium hydroxide, mix, add 2.5 mL ethyl acetate, centrifuge. Remove the organic layer and evaporate it to dryness at 40° under a stream of nitrogen, dissolve the residue in 200 μ L mobile phase, filter (0.45 μ m), inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 5 μ m Adsorbosphere C18 guard column

Column: 250 \times 4.6 5 μ m Adsorbosphere C18

Mobile phase: MeCN:water 26:74

Flow rate: 1

Injection volume: 50

Detector: UV 260

CHROMATOGRAM

Retention time: 6.8

Internal standard: 2-(4-chlorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol (9.5)

OTHER SUBSTANCES

Noninterfering: acyclovir, amikacin, amphotericin B, cefazolin, ceftazidime, clindamycin, gentamicin, metronidazole, piperacillin, trimethoprim, sulfamethoxazole

KEY WORDS

stability-indicating; water; saline; 5% dextrose

REFERENCE

Inagaki, K.; Tagaki, J.; Lor, E.; Lee, K.-J.; Nii, L.; Gill, M.A. Stability of fluconazole in commonly used intravenous antibiotic solutions. *Am.J.Hosp.Pharm.*, **1993**, *50*, 1206–1208

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: C18

Mobile phase: MeOH:25 mM sodium phosphate buffer 45:55, pH adjusted to 7.0 with phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 260

CHROMATOGRAM

Retention time: 6.1

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

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Thaler, F.; Bernard, B.; Tod, M.; Jednyak, C.P.; Petitjean, O.; Derome, P.; Loirat, P. Fluconazole penetration in cerebral parenchyma in humans at steady state. *Antimicrob.Agents Chemother.*, **1995**, *39*, 1154–1156 [brain; tissue; LOQ 150 ng/mL]

Burm, J.-P.; Choi, J.-S.; Jhee, S.S.; Chin, A.; Ulrich, R.W.; Gill, M.A. Stability of paclitaxel and fluconazole during simulated Y-site administration. *Am.J.Hosp.Pharm.*, **1994**, *51*, 2704–2706 [5% dextrose]

Flores-Murrieta, F.J.; Granados-Soto, V.; Hong, E. A simple and rapid method for determination of fluconazole in human plasma samples by high-performance liquid chromatography. *J.Liq. Chromatogr.*, **1994**, *17*, 3803–3811 [plasma; LOD 20 ng/mL]

Jenke, D.R. Drug binding by reservoirs in elastomeric infusion devices. *Pharm.Res.*, **1994**, *11*, 984–989 [saline; 5% dextrose]

Li, Z.W.; Guo, P.; Ye, L.M.; Hong, Z.; Wang, Y.S. Determination of fluconazole by direct injection of plasma and high performance liquid chromatography with column switching. *Yao Hsueh Hsueh Pao*, **1994**, *29*, 773–777

Madu, A.; Cioffe, C.; Mian, U.; Burroughs, M.; Tuomanen, E.; Mayers, M.; Schwartz, E.; Miller, M. Pharmacokinetics of fluconazole in cerebrospinal fluid and serum of rabbits: Validation of an animal model used to measure drug concentrations in cerebrospinal fluid. *Antimicrob.Agents Chemother.*, **1994**, *38*, 2111–2115 [pharmacokinetics; CSF; serum; plasma; rabbit; UK54373 (IS)]

Pompilio, F.M.; Fox, J.L.; Inagaki, K.; Burm, J.-P.; Jhee, S.; Gill, M.A. Stability of ranitidine hydrochloride with ondansetron hydrochloride or fluconazole during simulated Y-site administration. *Am.J.Hosp.Pharm.*, **1994**, *51*, 391–394 [saline; stability-indicating]

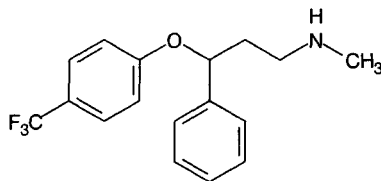
- Johnson, C.E.; Jacobson, P.A.; Pillen, H.A.; Woycik, C.L. Stability and compatibility of fluconazole and aminophylline in intravenous admixtures. *Am.J.Hosp.Pharm.*, **1993**, *50*, 703–706 [stability-indicating; UK-48-134 (IS); saline; 5% dextrose; non-interfering aminophylline]
- Yamreudeewong, W.; Lopez-Anaya, A.; Rappaport, H. Stability of fluconazole in an extemporaneously prepared oral liquid. *Am.J.Hosp.Pharm.*, **1993**, *50*, 2366–2367 [methyl p-hydroxybenzoate (IS); stability-indicating]
- Wallace, J.E.; Harris, S.C.; Gallegos, J.; Foulds, G.; Chen, T.J.; Rinaldi, M.G. Assay of fluconazole by high-performance liquid chromatography with a mixed-phase column. *Antimicrob.Agents Chemother.*, **1992**, *36*, 603–606
- Hosotsubo, K.K.; Hosotsubo, H.; Nishijima, M.K.; Okada, T.; Taenaka, N.; Yoshiya, I. Rapid determination of serum levels of a new antifungal agent, fluconazole, by high-performance liquid chromatography. *J.Chromatogr.*, **1990**, *529*, 223–228

Fluoxetine

Molecular formula: C₁₇H₁₈F₃NO

Molecular weight: 309.3

CAS Registry No.: 54910-89-3, 59333-67-4 (HCl)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + doxepin + 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: 16

Internal standard: doxepin (11.6)

OTHER SUBSTANCES

Extracted: amitriptyline, norfluoxetine, nortriptyline

KEY WORDS

plasma

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

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)) KH₂PO₄ 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:** 9.14**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

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

Interfering: amitriptyline, daunorubicin, etodolac, indomethacin, maprotiline, nortriptyline, tiocloamarol, tropatenine

KEY WORDSwhole blood; plasma

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: 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 the SPE cartridge, 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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: 6.5

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, haloperidol, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amidarone, 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, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: flurazepam, hydroxyethylflurazepam, 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: 1 mL Serum or plasma + 30 μ L trimipramine in MeOH + 200 μ L 0.33 M NaOH, shake 5 s, add 7 mL n-hexane:iso-amyl alcohol 985:15, shake 20 min, centrifuge at 2100 g for 5 min. Remove organic phase and add 200 μ L 0.1 M HCl to it, shake for 1 min, discard organic phase, inject 30 μ L of aqueous phase.

HPLC VARIABLES

Guard column: 10 mm 10 μ m Bischoff C18

Column: 125 \times 4.5 μ m Ecotube Nucleosil C8

Mobile phase: MeCN:water:diethylamine:PicB5 370:630:0.4:25 (PicB5 is water-MeOH-1-pentanesulfonic acid.)

Column temperature: 55

Flow rate: 1.7

Injection volume: 30

Detector: UV 230

CHROMATOGRAM

Retention time: 5.6

Internal standard: trimipramine (8.4)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: norfluoxetine

Noninterfering: alprazolam, amitriptyline, bromazepam, clomipramine, clorazepate, desipramine, diazepam, flunitrazepam, fluvoxamine, imipramine, lorazepam, nortriptyline, oxazepam, triazolam

KEY WORDS

serum; plasma

REFERENCE

el Maanni, A.; Combourieu, I.; Bonini, M.; Creppy, E.E. Fluoxetine, an antidepressant, and norfluoxetine, its metabolite, determined by HPLC with a C_8 column and ultraviolet detection. *Clin. Chem.*, **1993**, *39*, 1749–1750

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 μ L Serum + 50 μ L 5 μ g/mL protriptyline in 5% potassium bicarbonate + 700 μ L 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 μ L MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluate, inject a 50 μ L aliquot of the eluate.

HPLC VARIABLES

Guard column: 15 mm 7 μ m Brownlee RP-8

Column: 150 \times 4.6 5 μ m 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 226

CHROMATOGRAM

Retention time: 10.9

Internal standard: protriptyline (6.6)

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, desipramine, doxepin, fluvoxamine, imipramine, maprotiline, nortriptyline, trimipramine

Interfering: desmethylclomipramine

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: Add 10 μL 20 $\mu\text{g}/\text{mL}$ 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 \times 4.6 10 μm Hypersil MOS C8; B 20 \times 4.6 5 μm Hypersil CPS CN + 250 \times 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: 8.7

Internal standard: oxaprotiline (9.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: clozapine, desipramine, doxepin, fluvoxamine, imipramine, maprotiline, metoclopramide, norfluoxetine, nortriptyline

Noninterfering: carbamazepine, chlordiazepoxide, clobazam, diazepam, flurazepam, fluspirilene, haloperidol, lorazepam, nitrazepam, nordiazepam, oxazepam, perazine, pimozide, spiroperidol, trifluoperidol

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: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 $\mu\text{g}/\text{mL}$ 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 \times 4.6 5 μm Supelcosil LC-18

Mobile phase: MeCN:25 mM KH_2PO_4 75:25 containing 500 $\mu\text{L/L}$ orthophosphoric acid and 600 $\mu\text{L/L}$ n-butylamine

Flow rate: 2

Injection volume: 25-40

Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM

Retention time: 10.4

Internal standard: maprotiline (12.8)

Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxapine, clovoxamine, desipramine, fenfluramine, fluvoxamine, norfluoxetine, nortriptyline, propranolol, protriptyline, sertraline

Noninterfering: amitriptyline, atenolol, bupropion, carbamazepine, chlordiazepoxide, citalopram, clomipramine, clozapine, cyclobenzaprine, doxepin, imipramine, loxapine, metoprolol, mianserin, moclobemide, nomifensine, pindolol, thioridazine, tranlycypromine, trazodone, trimipramine

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 Plasma + 160 μL 10 $\mu\text{g/mL}$ clomipramine in MeOH, vortex, add 2 mL 1 M NaOH, vortex, add 5 mL n-hexane:isoamyl alcohol 99:1, rotate for 5 min, centrifuge for 10 min. Remove organic layer and add it to 200 μL 0.05% phosphoric acid, rotate for 5 min, centrifuge for 10 min, remove lower aqueous layer and inject a 25-50 μL aliquot of it.

HPLC VARIABLES

Guard column: Bondapak/Corasil C18

Column: 300 \times 4.6 μm Bondapak C18

Mobile phase: MeCN:50 mM KH_2PO_4 adjusted to pH 4.7 with KOH 40:60

Column temperature: 50

Flow rate: 2

Injection volume: 25-50

Detector: UV 214

CHROMATOGRAM

Retention time: 7

Internal standard: clomipramine (9)

Limit of detection: 6 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxapine, chlordiazepoxide, chlorpromazine, cimetidine, desipramine, diazepam, doxepin, flurazepam, imipramine, lorazepam, norfluoxetine, nortriptyline, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, secobarbital, thioridazine, trifluoperazine

Noninterfering: acetaminophen, codeine, meperidine

Interfering: amitriptyline, propoxyphene

KEY WORDS

plasma

REFERENCE

Wong, S.H.; Dellafera, S.S.; Fernandes, R.; Kranzler, H. Determination of fluoxetine and norfluoxetine by high-performance liquid chromatography. *J. Chromatogr.*, **1990**, *499*, 601-608

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Serum + 50 μ L 1 mg/mL reduced haloperidol in water + 2 mL 0.5 M NaH_2PO_4 adjusted to pH 10 with 10 M NaOH, vortex 3-5 s, add 5 mL hexane:isoamyl alcohol 97:3, shake 20 min, centrifuge at 1000 g for 10 min. Remove organic layer and add 1 mL 0.1 M HCl to it, shake for 20 min, centrifuge for 10 min, discard organic layer. Add 1 mL 0.2 M NaOH to aqueous layer, add 5 mL hexane:isoamyl alcohol 97:3, shake for 20 min, centrifuge for 10 min. Remove organic layer, add 1 drop of 0.3 M HCl in MeOH, evaporate under nitrogen at 40°, reconstitute with 250 μ L mobile phase, vortex, inject 100 μ L aliquot.

HPLC VARIABLES**Guard column:** C18 Guard-Pak (Waters no. 88070)**Column:** Nova-Pak phenyl (Waters no. 10656)**Mobile phase:** MeCN:buffer 40:60 (Buffer was 600 mL water + 1 mL triethylamine, adjusted to pH 5.5 with acetic acid.)**Flow rate:** 1.7**Injection volume:** 100**Detector:** UV 226

CHROMATOGRAM**Retention time:** 7.3**Internal standard:** reduced haloperidol (3.9)**Limit of detection:** 15 ng/mL

OTHER SUBSTANCES

Simultaneous: alprazolam, amitriptyline, amoxapine, chlordiazepoxide, chlorimipramine, clonazepam, demoxepam, diazepam, doxepin, halazepam, haloperidol, lorazepam, maprotiline, norfluoxetine, nortriptyline, oxazepam, temazepam, trazodone, trimipramine

Interfering: desipramine, imipramine, loxapine, protriptyline

KEY WORDS

serum

REFERENCE

Orsulak, P.J.; Kenney, J.T.; Debus, J.R.; Crowley, G.; Wittman, P.D. Determination of the antidepressant fluoxetine and its metabolite norfluoxetine in serum by reversed-phase HPLC with ultraviolet detection. *Clin. Chem.*, **1988**, *34*, 1875-1878

SAMPLE**Matrix:** blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μ g cyanopramine + 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 μ L 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μ L aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μ g cyanopramine + 500 μ L 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 μ L 0.2% phosphoric acid, agitate

gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m RP-18 Newguard (Applied Biosystems)

Column: 100 \times 4.6 5 μ m Brownlee Spheri-5 RP-18

Mobile phase: MeCN:100 mM NaH₂PO₄:diethylamine 40:57.5:2.5

Flow rate: 2

Injection volume: 30

Detector: UV 220

CHROMATOGRAM

Retention time: 6.70

Internal standard: cianopramine (8.93)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: 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, tranlycypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: amoxapine

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: Homogenize (Brinkman Polytron) tissue with 5-10 volumes of water. 500 μ L Plasma or tissue homogenate + 500 μ L water + 50 μ L 2 μ g/mL IS in water + 100 μ L 1 M NaOH, vortex gently, add 5 mL hexane:butanol 99.7:0.3, shake mechanically at 125-150 cycles/min for 30 min, centrifuge at 2000 g for 15 min. Remove the organic layer and mix it with 100 μ L 200 μ M R-(–)-1-(1-naphthyl)ethyl isocyanate in hexane, evaporate to dryness at 50-55° over 20-30 min, dry more vigorously when all the hexane is gone, reconstitute with 200 μ L mobile phase, inject a 75 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Apex silica (Jones Chromatography)

Mobile phase: Isooctane:THF 70:30

Column temperature: 35

Flow rate: 1

Injection volume: 75

Detector: F ex 218 em 333

CHROMATOGRAM

Retention time: 8.3 (S), 9.3 (R)

Internal standard: S-nornisoxetine (15)

Limit of detection: 5 ng/mL (plasma); 25 ng/g (tissue)

OTHER SUBSTANCES

Extracted: metabolites, norfluoxetine

KEY WORDS

chiral; derivatization; normal phase; plasma; silylate all glassware

REFERENCE

Potts, B.D.; Parli, C.J. Analysis of the enantiomers of fluoxetine and norfluoxetine in plasma and tissue using chiral derivatization and normal-phase liquid chromatography. *J.Liq.Chromatogr.*, **1992**, *15*, 665–681

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 2 mg/mL solution of fluoxetine hydrochloride in mobile phase, inject a 10 μ L solution.

HPLC VARIABLES

Column: 150 \times 4.6 3 μ m Exsil 100 \AA /ODS-B octadecylsilane (Keystone)

Mobile phase: MeCN:THF:buffer 15:10:75 (Buffer was 50 mM ammonium acetate adjusted to pH 5.75 with 50 mM acetic acid.)

Flow rate: 1

Injection volume: 10

Detector: UV 214

CHROMATOGRAM

Retention time: 31.9

OTHER SUBSTANCES

Simultaneous: impurities, meta isomer

REFERENCE

Lacroix, P.M.; Yat, P.N.; Lovering, E.G. Liquid chromatographic methods for fluoxetine hydrochloride, its *meta* isomer, and related compounds in raw materials. *J.AOAC Int.*, **1995**, *78*, 334–339

SAMPLE

Matrix: bulk

Sample preparation: Reflux 1.23 g fluoxetine with 788 mg (R)-(-)-1-(1-naphthyl)ethyl isocyanate in 25 mL toluene for 2 h, evaporate to dryness under reduced pressure, reconstitute, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 silica (IBM)

Mobile phase: Dichloromethane:MeOH 99.75:0.25

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 6.69 (S), 7.52 (R)

KEY WORDS

derivatization; chiral; normal phase

REFERENCE

Robertson, D.W.; Krushinski, J.H.; Fuller, R.W.; Leander, J.D. Absolute configurations and pharmacological activities of the optical isomers of fluoxetine, a selective serotonin-uptake inhibitor. *J.Med.Chem.*, **1988**, *31*, 1412-1417

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 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax cyano special

Mobile phase: MeCN:buffer 50:50 (Buffer was 1% triethylamine, pH adjusted to 6 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: benzoic acid

KEY WORDS

syrup; elixir; stability-indicating

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: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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), 7.07 (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, colchi-

cine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, 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, methyl-dopa, 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, pimizide, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 × 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: 11.460

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Asch, 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: tissue

Sample preparation: Homogenize 1 mL liver in 5 mL water. Centrifuge 1 mL homogenate, add the supernatant to 1.2 µg clomipramine, add 75 µL MeOH, add 75 µL MeCN, add 100 µL 1 M HCl, vortex, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18

Mobile phase: MeCN:50 mM potassium phosphate buffer 35:65

Flow rate: 1.3

Detector: UV 226

CHROMATOGRAM

Internal standard: clomipramine

OTHER SUBSTANCES

Extracted: metabolites, norfluoxetine

Also analyzed: sertraline

KEY WORDS

mouse; liver

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

ANNOTATED BIBLIOGRAPHY

Hubmann, M.R.; Waschler, R.; Moll, W.; Conca, A.; König, P. Simultaneous drug monitoring of citalopram, clozapine, fluoxetine, maprotiline, and trazodone by HPLC analysis (Abstract 41). *Ther.Drug Monit.*, **1995**, *17*, 393 [simultaneous citalopram, clozapine, maprotiline, trazodone; LOQ 50 ng/mL]

Joron, S.; Robert, H. Simultaneous determination of antidepressant drugs and metabolites by HPLC. Design and validation of a simple and reliable analytical procedure. *Biomed.Chromatogr.*, **1994**, *8*, 158–164 [simultaneous amineptine, amitriptyline, amoxapine, clomipramine, demexiptiline, desipramine, dosulepine, doxepin, doxepine, fluvoxamine, imipramine, maprotiline, medifloxamine, mianserine, opipramol, quinupramine, tianeptine, toloxatone, trazodone, trimipramine, viloxazine; LOQ 3-17 ng/mL; plasma]

Thomare, P.; Wang, K.; Van Der Meersch-Mougeot, V.; Diquet, B. Sensitive micromethod for column liquid chromatographic determination of fluoxetine and norfluoxetine in human plasma. *J.Chromatogr.*, **1992**, *583*, 217–221 [plasma; extracted metabolites; LOD 2 ng/mL; simultaneous amineptine, amitriptyline, chlordiazepoxide, chlorpromazine, clomipramine, clonazepam, clorazepate, desipramine, diazepam, doxepin, flunitrazepam, fluvoxamine, imipramine, levomepromazine, lorazepam, loxapine, maprotiline, mefloquine, nortriptyline, oxazepam, thioridazine]

Peyton, A.L.; Carpenter, R.; Rutkowski, K. The stereospecific determination of fluoxetine and norfluoxetine enantiomers in human plasma by high-pressure liquid chromatography (HPLC) with fluorescence detection. *Pharm.Res.*, **1991**, *8*, 1528–1532

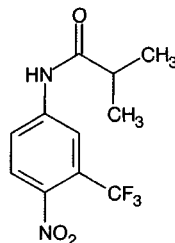
Gupta, R.N.; Steiner, M. Determination of fluoxetine and norfluoxetine in serum by liquid chromatography with fluorescence detection. *J.Liq.Chromatogr.*, **1990**, *13*, 3785–3798 [extracted norfluoxetine; serum; fluorescence detection; SPE; protriptyline (IS); simultaneous amitriptyline, nortriptyline]

Flutamide

Molecular formula: C₁₁H₁₁F₃N₂O₃

Molecular weight: 276.2

CAS Registry No.: 13311-84-7



SAMPLE

Matrix: blood

Sample preparation: 150 μ L Plasma + 150 μ L MeCN, vortex for 15 s, centrifuge at 13000 g for 10 min, inject a 50 μ L aliquot of the supernatant

HPLC VARIABLES

Guard column: 30 mm long 40-50 μ m pellicular C18 (Replace guard column and prefilter before each run.)

Column: 150 \times 3.2 \times 3 μ m Sphere 3 ODS C18 (Phenomenex)

Mobile phase: MeCN:1% acetic acid 50:50, pH 2.9 (dog) or MeCN:MeOH:water 30:20:50 (human)

Flow rate: 0.5

Injection volume: 50

Detector: UV 300

CHROMATOGRAM

Retention time: 7.4

Limit of detection: 11.27 ng/mL

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

dog; plasma; pharmacokinetics; human

REFERENCE

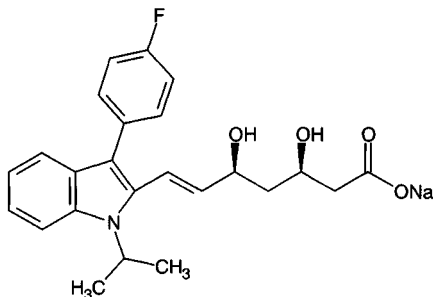
Farthing, D.; Sica, D.; Fakhry, I.; Walters, D.L.; Cefali, E.A.; Allan, G. Determination of flutamide and hydroxyflutamide in dog plasma by a sensitive high performance liquid chromatography method utilizing mid-bore chromatography. *Biomed.Chromatogr.*, **1994**, *8*, 251-254

Fluvastatin

Molecular formula: C₂₄H₂₆FNO₄

Molecular weight: 410.5

CAS Registry No.: 93957-55-2 (fluvastatin sodium)



SAMPLE

Matrix: blood

Sample preparation: Dilute with an equal volume of water, precipitate with two 10 mL portions of acetone:MeOH 5:2, collect the supernatant, reduce it in volume, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm fatty acid (Waters)

Mobile phase: Gradient. Buffer:MeOH 100:0 for 2 min, to 72:18 over 25 min, maintain at 72:28 for 18 min, to 62:38 over 19 min, maintain at 62:38 for 6 min, to 59.7:40.3 over 4 min, to 59.2:40.8 over 2 min, maintain at 59.2:40.8 for 2 min, to 58:42 over 2 min, maintain at 58:42 for 2 min, to 54:46 over 4 min, to 45:55 over 4 min, maintain at 45:55 for 2 min, to 20:80 over 6 min, maintain at 20:80 over 6 min, return to initial conditions over 4 min.

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 85

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; human; rat; hamster

REFERENCE

Dain, J.G.; Fu, E.; Gorski, J.; Nicoletti, J.; Scallen, T.J. Biotransformation of fluvastatin sodium in humans. *Drug Metab.Dispos.*, **1993**, *21*, 567-572

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL MeCN, mix on a Maxi-Mix for 5 s, add 1 mL 300 ng/mL IS in water, add 2 mL phosphate buffer, add 10 mL MTBE, shake horizontally on a platform shaker at 200 cycles/min for 15 min, centrifuge at 700 g for 5 min. Remove the upper organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 400 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeOH:13 mM tetrabutylammonium fluoride 60:40

Column temperature: 50

Injection volume: 200

Detector: F ex 305 em 380

CHROMATOGRAM

Retention time: 9.2

Internal standard: ([R*, S*)-(E)-](±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-methyl-6-heptenoic acid, monosodium salt (Sandoz 63-267, 6-methyl-fluvastatin) (12.8)

Limit of quantitation: 1 ng/mL

KEY WORDS

plasma; protect from light; pharmacokinetics

REFERENCE

Kalafsky, G.; Smith, H.T.; Choc, M.G. High-performance liquid chromatographic method for the determination of fluvastatin in human plasma. *J.Chromatogr.*, **1993**, *614*, 307–313

SAMPLE

Matrix: blood

Sample preparation: Adjust pH of 500 µL blood to 7, extract with MTBE. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Supelcosil LC-18

Mobile phase: MeOH:water 60:40 containing 5 mL/L tetrabutylammonium fluoride

Column temperature: 50

Detector: F ex 305 em 380

CHROMATOGRAM

Internal standard: sodium 3,5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indole-2-yl]-6-methylhept-6-enoate

Limit of detection: 1 ng/mL

KEY WORDS

pharmacokinetics; rabbit

REFERENCE

Tse, F.L.; Labbadia, D. Absorption and disposition of fluvastatin, an inhibitor of HMG-CoA reductase, in the rabbit. *Biopharm.Drug Dispos.*, **1992**, *13*, 285–294

SAMPLE

Matrix: blood

Sample preparation: Adjust pH of 500 µL blood to 7, extract with MTBE. Remove the organic layer and evaporate it to dryness, reconstitute the residue in MeCN:5 mM pH 6.5 hexyltriethylammonium phosphate 5:95, inject an aliquot.

HPLC VARIABLES

Column: C8

Mobile phase: MeCN:5 mM pH 6.5 hexyltriethylammonium phosphate 40:60

Column temperature: 50

Detector: F ex 305 em 380

CHROMATOGRAM

Limit of detection: 2 ng/mL

KEY WORDS

pharmacokinetics; mouse; dog; monkey

REFERENCE

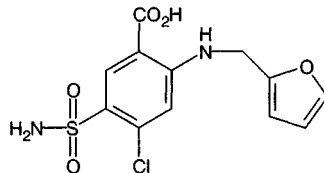
Tse, F.L.; Smith, H.T.; Ballard, F.H.; Nicoletti, J. Disposition of fluvastatin, an inhibitor of HMG-CoA reductase, in mouse, rat, dog, and monkey. *Biopharm. Drug Dispos.*, **1990**, *11*, 519–531

Furosemide

Molecular formula: C₁₂H₁₁ClN₂O₅S

Molecular weight: 330.7

CAS Registry No.: 54-31-9



SAMPLE

Matrix: bile

Sample preparation: 2 mL Bile + 1 mL pH 5.0 phosphate buffer, filter (0.5 μm). Remove a 1 mL aliquot and add it to 500 μL 20 μg/mL piretanide, inject a 10-50 μL aliquot. (Hydrolyze glucuronide by heating 2 mL bile with 1 mL 1000 U/mL β-glucuronidase in 100 mM pH 5.0 acetate buffer at 37° for 2 h, proceed as above.)

HPLC VARIABLES

Column: 150 × 6 5 μm Shim-pack CLC-ODS (Shimadzu)

Mobile phase: Gradient. A was MeCN:water 20:80 containing 0.3% acetic acid. B was MeCN:water 80:20 containing 0.3% acetic acid. A:B 90:10 for 3 min, to 60:40 over 7 min, maintain at 60:40 for 5 min, to 40:60 over 3 min, to 60:40 over 2 min, to 90:10 over 10 min.

Column temperature: 40

Injection volume: 10-50

Detector: F ex 345 em 415

CHROMATOGRAM

Retention time: 15

Internal standard: piretanide (21)

Limit of detection: 5 ng/mL

KEY WORDS

pharmacokinetics

REFERENCE

Sekikawa, H.; Yagi, N.; Oda, K.; Kenmotsu, H.; Takada, M.; Chen, H.-f.; Lin, E.T.; Benet, L.Z. Biliary excretion of furosemide glucuronide in rabbits. *Biol.Pharm.Bull.*, **1995**, *18*, 447-453

SAMPLE

Matrix: blood

Sample preparation: 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:2.5 mM pH 5 ammonium phosphate 1:50; B MeCN:2.5 mM pH 2.5 ammonium phosphate 31:69; C MeCN:water 50:50

Flow rate: A 1.2; B 1; C 1.5

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 19.7

Limit of detection: 100 ng/mL

KEY WORDS

plasma; dog; beagle; column-switching

REFERENCE

Matsuura, A.; Nagayama, T.; Kitagawa, T. Automated high-performance liquid chromatographic method for determination of furosemide in dog plasma. *J.Chromatogr.*, **1993**, *617*, 339–343

SAMPLE

Matrix: blood

Sample preparation: 25 μL Plasma + 100 μL 10 $\mu\text{g}/\text{mL}$ naproxen in MeCN, vortex 30 s, centrifuge at 11000–12300 g for 7 min. Remove supernatant and evaporate it under air at 55°. Dissolve residue in 50 μL mobile phase and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeCN:80 mM pH 2.0 orthophosphoric acid 46:54

Flow rate: 1.1

Injection volume: 20

Detector: F ex 270 em 410

CHROMATOGRAM

Retention time: 6.5

Internal standard: naproxen (11.5)

Limit of detection: 10 ng/mL

Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products

Noninterfering: amikacin, amoxicillin, dexamethasone, gentamicin, indomethacin, morphine, phenobarbital, theophylline, vitamins

KEY WORDS

plasma; microscale; neonatal

REFERENCE

Sidhu, J.S.; Charles, B.G. Simple microscale high-performance liquid chromatographic method for determination of furosemide in neonatal plasma. *J.Chromatogr.*, **1993**, *612*, 161–165

SAMPLE

Matrix: blood

Sample preparation: Condition an Analytichem C2 ethyl sorbent SPE cartridge with 1 mL MeCN and 1 mL buffer. 25 μL Plasma + 1 mL buffer, add to the SPE cartridge, wash with 1 mL buffer, blow dry with nitrogen for 1 min, elute cartridge directly onto column (Varian AASP system). (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.0 with concentrated phosphoric acid.)

HPLC VARIABLES

Guard column: 3 \times 4.6 30 μm C18 Alltech pellicular packing

Column: 150 \times 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:10 mM KH_2PO_4 adjusted to pH 3.0 with concentrated phosphoric acid 30:70

Flow rate: 1.5

Detector: F ex 272 em 410

CHROMATOGRAM

Retention time: 9.1

Internal standard: metolazone (8.3)

Limit of detection: 1.8 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, bumetanide, chlorothiazide, chlorthalidone, hydrochlorothiazide, ibuprofen, salicylic acid

KEY WORDS

plasma; SPE; better results with external standard

REFERENCE

Farthing, D.; Karnes, T.; Gehr, T.W.; March, C.; Fakhry, I.; Sica, D.A. External-standard high-performance liquid chromatographic method for quantitative determination of furosemide in plasma by using solid-phase extraction and on-line elution. *J.Pharm.Sci.*, **1992**, *81*, 569–571

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 250 μ L 8.5 M acetic acid, mix, add 250 μ L 125 mM sodium dodecylsulfate, mix 5 s, add 100 μ L 40 μ g/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 μ L mobile phase, inject 100 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 2.5 μ m Nucleosil 100 C18

Column: 100 \times 3.5 μ m 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: 4.5

Internal standard: naproxen (12)

Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES

Simultaneous: amiloride

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

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 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 3 μ m 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: 5.05

Internal standard: naproxen (3.5)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: bumetanide

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, CSF

Sample preparation: 200 μ L Serum, plasma, or CSF + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 \times 2.1 40 μ m preparative grade C18 (Analytichem); B 250 \times 4.6 10 μ m 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: 9.12

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

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

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: Filter (0.45 μm) urine or plasma. Mix plasma filtrate with an equal volume of 50 mM pH 8.0 Tris-sulfuric acid buffer containing 0.1 mM zinc acetate and 40 mM sodium dodecyl sulfate. Inject a 50 μL aliquot of the urine filtrate or the diluted plasma onto column A with mobile phase A, elute to waste with mobile phase A, after 5 min backflush the contents of column A onto column B with mobile phase B, after 3 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 5 min before next injection.

HPLC VARIABLES

Column: A 10 \times 4.6 carbonic anhydrase (Prepare by adding 3 g aminopropyl silica from a Sep-Pak NH₂ SPE cartridge to 30 mL 100 mg/mL N, N'-disuccinimidyl carbonate in MeCN in portions over 30 min with gentle mixing, mix for 3 h, filter (G-5 glass), wash the solid 5 times with 50 mL portions of MeCN. Add 100 mg activated gel to 2 mL 200 mM pH 8.0 phosphate buffer containing 1 M NaCl, degas by sonicating under aspirator vacuum, add 2 mL 2.5 mg/mL carbonic anhydrase in water, shake at room temperature for 4 h, centrifuge, discard the supernatant, suspend the gel in 50 mM pH 8.0 Tris-sulfuric acid buffer, slurry pack into column. Store in 50 mM pH 8.0 Tris-sulfuric acid buffer containing 0.1 mM zinc acetate when not in use.); B 150 \times 4.6 Cosmosil 5C18-AR (Nakarai Tesque)

Mobile phase: A 50 mM pH 8.0 Tris-sulfuric acid buffer containing 0.1 mM zinc acetate; B Gradient. MeCN:100 mM pH 5.2 acetate buffer 10:90 containing 500 mM NaCl for 3 min then MeCN:100 mM pH 5.2 acetate buffer 23:77 containing 500 mM NaCl (step gradient). (Increase in gradient occurs at the same time as column A is removed from the circuit.)

Flow rate: 1

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Also analyzed: acetazolamide, chlorothiazide, chlorthalidone, hydrochlorothiazide

Noninterfering: acetaminophen, bumetanide, caffeine, phenylbutazone, salicylic acid, sulfamerazine, sulfamethiazole, sulfamethoxazole, sulfamonomethoxine, sulfisoxazole, sulfisomidine, theophylline, tolbutamide, warfarin

KEY WORDS

plasma; column-switching

REFERENCE

Ohta, T.; Takamiya, I.; Takitani, S. Carbonic anhydrase-immobilized precolumn for selective on-line sample pretreatment in high-performance liquid chromatographic determination of certain sulphonamide drugs. *Biomed.Chromatogr.*, **1994**, *8*, 184–188

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μL Plasma + 100 μL MeCN, centrifuge at 3000 g for 5 min, inject 20 μL aliquot of supernatant. Urine. Dilute urine 1:1 with water and inject 20 μL .

HPLC VARIABLES

Guard column: 75 \times 2.1 pellicular reversed phase (Chrompack cat. no. 28653)

Column: 250 \times 4.6 5 μm Cp Spherisorb ODS

Mobile phase: Gradient. MeCN:0.5% pH 2.1 orthophosphoric acid (98%), from 5:95 to 41:59 over 30 min, stay at 41:59 for 5 min, return to 5:95 over 5 min, equilibrate for 2 min before next injection.

Flow rate: 1.2

Injection volume: 20

Detector: F ex 345 em 405

CHROMATOGRAM

Retention time: 28.77

Limit of detection: 5 ng/mL

Limit of quantitation: 7 ng/mL (plasma); 100 ng/mL (urine)

OTHER SUBSTANCES

Simultaneous: metabolites, glucuronides

KEY WORDS

plasma

REFERENCE

Vree, T.B.; Van den Biggelaar-Martea, M.; Verwey-van Wissen, C.P.W.G.M. Determination of furosemide with its acyl glucuronide in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis with fluorescence detection. Preliminary pharmacokinetics and effect of probenecid. *J.Chromatogr.B*, **1994**, 655, 53-62

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 NaH₂PO₄ 20:80 adjusted to pH 4.2 with phosphoric acid

Flow rate: 1.2

Injection volume: 20

Detector: UV 228

CHROMATOGRAM

Retention time: 4.52

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: formulations, urine

Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 30 min, sonicate for 5 min, filter (Albet 242 paper), wash solid with MeOH, make up filtrate to 50 mL with MeOH, inject a 20 μL aliquot. Urine. Adjust pH of 2 mL urine to 10.0 with 2 M KOH, add 1.5 mg NaCl, add 4 mL ethyl acetate, shake for 10 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 2 mL mobile phase, sonicate, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:water 30:70 containing 5 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH adjusted to 5.5

Flow rate: 1

Injection volume: 20

Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1300 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 5 min.)

CHROMATOGRAM

Retention time: 6.70

Limit of detection: 15 ng/mL

OTHER SUBSTANCES

Extracted: triamterene

KEY WORDS

tablets; pharmacokinetics

REFERENCE

Barroso, M.B.; Alonso, R.M.; Jiménez, R.M. Simultaneous determination of the diuretics triamterene and furosemide in pharmaceutical formulations and urine by HPLC-EC. *J.Liq.Chrom.Rel.Technol.*, 1996, 19, 231-246

SAMPLE

Matrix: formulations, urine

Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 20 min, filter, wash solid with MeOH, dilute filtrate with mobile phase, inject a 20 μ L aliquot. Urine. 2 mL Urine + 2 mL 1 M pH 3.25 KH_2PO_4 + 4 mL ethyl acetate, vortex for 20 min, centrifuge at 734 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:water 40:60 containing 5 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH adjusted to 4.25

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1200 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 15 min.)

CHROMATOGRAM

Retention time: 7.7

Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES

Extracted: pirtanide

KEY WORDS

tablets; pharmacokinetics

REFERENCE

Barroso, M.B.; Jiménez, R.M.; Alonso, R.M.; Ortiz, E. Determination of piretanide and furosemide in pharmaceuticals and human urine by high-performance liquid chromatography. *J.Chromatogr.B*, **1996**, *675*, 303-312

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:water 40:60 containing 10 mM KH₂PO₄

Flow rate: 1

Detector: UV 264

KEY WORDS

rat; rabbit; pharmacokinetics

REFERENCE

Sinko, P.J.; Hu, P.; Wacławski, 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: 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: 5.78 (A), 5.03 (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, 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, 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, methyl-dopa, 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, sulfapyrazone, 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

some 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 μ L aliquot.

HPLC VARIABLES

Guard column: 5 \times 4 10 μ m LiChrosorb RP-8

Column: 100 \times 4.6 5 μ m 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 \times 0.3 mm coil of PTFE tubing irradiated at 254 nm with a Sylvania GTE 8 W low-pressure lamp to the detector.

OTHER SUBSTANCES

Also analyzed: bendroflumethiazide, butizide, chlorthalidone, ethacrynic acid, 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: Prepare a 1-10 μ g/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Hypersil SCX/C18

Mobile phase: MeCN:25 mM pH 3 Na₂HPO₄ 50:50

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.42

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, 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

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 KH₂PO₄ and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 227, 266

CHROMATOGRAM

Retention time: 4.7

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, 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 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, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isosuxiprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methylidopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niftumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiaabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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: 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 NaH₂PO₄ + 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: 8.7

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, 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

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 NaH₂PO₄·H₂O in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 16.4

Limit of detection: 1 μg/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, 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 μ L 100 μ g/mL 7-propyltheophylline in MeOH + 200 μ L 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 μ L MeCN:water 15:85 and inject 20 μ L 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 \times 4.6 3 μ m 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: 5.9

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene, 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: 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 μ L MeCN/water, inject a 10-20 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4 5 μ m 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; UV 270

CHROMATOGRAM

Retention time: 6.3

Limit of detection: 500 ng (by MS)

OTHER SUBSTANCES

Extracted: amiloride, bendroflumethiazide, benzthiazide, chlorthalidone, triamterene

REFERENCE

Ventura, R.; Fraisse, D.; Becchi, M.; Paise, 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 + 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: UV 230

CHROMATOGRAM

Retention time: 6.2

Internal standard: bendroflumethiazide (8.6)

OTHER SUBSTANCES

Extracted: bumetanide, canrenone, cyclopenthiazide, etozolin, piretanide

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: 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 KH₂PO₄:Na₂HPO₄ 99:1, solid buffer II was NaHCO₃:K₂CO₃ 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 NaH₂PO₄ 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:** 12.16 (A); 12.9 (B)**Internal standard:** β -hydroxyethyltheophylline (3.7 (A), 4.4 (B))**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Extracted:** acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, canrenone, chlorothiazide, chlorthalidone, cyclothiazide, dichlorphenamide, ethacrynic acid, flumethiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, probenecid, quinethazone, spironolactone, triamterene, trichloromethiazide**Noninterfering:** acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil**Interfering:** metolazone

REFERENCECooper, 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 + 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 \times 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:** 5.0**Limit of quantitation:** 1 $\mu\text{g}/\text{mL}$

OTHER SUBSTANCES**Extracted:** bendroflumethiazide, bumetanide, chlorothiazide, chlorthalidone, clopamide, cyclopenthiiazide, hydrochlorothiazide, mefruside, methyclothiazide, metolazone, quinethazone**Simultaneous:** clorexolone, ethacrynic acid, indapamide**Noninterfering:** albuterol, allopurinol, alprenolol, aspirin, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyl dopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCEFullinaw, R.O.; Bury, R.W.; Moulds, R.F.W. Liquid chromatographic screening of diuretics in urine. *J. Chromatogr.*, **1987**, *415*, 347–356

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- Vree, T.B.; Van den Biggelaar-Martea, M.; Verwey-van Wissen, C.P. Determination of furosemide with its acyl glucuronide in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis with fluorescence detection. Preliminary pharmacokinetics and effect of probenecid. *J.Chromatogr.B*, **1994**, *655*, 53–62
- Herráez-Hernández, R.; Campíns-Falcó, P.; Sevillano-Cabeza, A. Improved screening procedure for diuretics. *J.Liq.Chromatogr.*, **1992**, *15*, 2205–2224 [LOD 10-1000 ng/mL; gradient; urine; hydroxymethyltheophylline (IS); extracted acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, hydrochlorothiazide, probenecid, spironolactone, triamterene]
- Campíns-Falcó, P.; Herráez-Hernández, R.; Sevillano-Cabeza, A. Solid-phase extraction techniques for assay of diuretics in human urine samples. *J.Liq.Chromatogr.*, **1991**, *14*, 3575–3590 [urine; SPE; hydroxyethyltheophylline (IS); extracted acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, hydrochlorothiazide, probenecid, spironolactone, triamterene]
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- Santasia, C.T. Direct injection analysis of diuretic and anti-inflammatory drugs on a shielded hydrophobic phase column. *J.Liq.Chromatogr.*, **1990**, *13*, 2605–2631 [direct injection; serum; gradient; horse; extracted hydrochlorothiazide, oxyphenbutazone, phenylbutazone]
- Berthod, A.; Asensio, J.M.; Laserna, J.J. Micellar liquid chromatography for rapid screening of illegal drugs in sport. *J.Liq.Chromatogr.*, **1989**, *12*, 2621–2634 [fluorescence detection; UV detection; also bumetanide, caffeine, chlorthalidone, dihydrochlorothiazide, ephedrine, methyltestosterone, oxandrolone, propranolol, spironolactone, testosterone]
- Radeck, W.; Heller, M. Improved method for the determination of furosemide in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1989**, *497*, 367–370
- Russel, F.G.; Tan, Y.; Van Meijel, J.J.; Gribnau, F.W.; Van Ginneken, C.A. Solid-phase extraction of furosemide from plasma and urine and subsequent analysis by high-performance liquid chromatography. *J.Chromatogr.*, **1989**, *496*, 234–241
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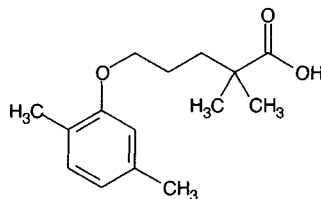
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- Nation, R.L.; Peng, G.W.; Chiou, W.L. Quantitative analysis of furosemide in micro plasma volumes by high-performance liquid column chromatography. *J. Chromatogr.*, **1979**, *162*, 88–93 [plasma; fluorescence detection; non-interfering metabolites, acetaminophen, ampicillin, caffeine, digoxin, ephedrine, phenacetin, phenobarbital, phenytoin, salicylic acid, tetracycline, theobromine, theophylline; LOD 100 ng/mL]

Gemfibrozil

Molecular formula: C₁₅H₂₂O₃

Molecular weight: 250.3

CAS Registry No.: 25812-30-0



SAMPLE

Matrix: blood

Sample preparation: Adjust pH of plasma to 4-5 with phosphoric acid (about 30 μ L phosphoric acid per 4 mL of plasma). 500 μ L Acidified plasma + 100 μ L 2 μ g/mL flurbiprofen in MeCN:water 30:70 + 1.3 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and add it to 800 μ L 1 M pH 3.0 glycine buffer and 4 mL ethyl acetate, shake horizontally at 70 rpm for 15 min, centrifuge at 1000 g for 15 min. Remove the organic layer and evaporate it to dryness in an evacuated centrifuge, reconstitute the residue in 250 μ L mobile phase, inject a 10-100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere cyano

Mobile phase: MeCN:10 mM tetrabutylammonium sulfate 28:72, final apparent pH adjusted to 3.5

Flow rate: 1

Injection volume: 10-100

Detector: F ex 284 em 316

CHROMATOGRAM

Retention time: 20.9

Internal standard: flurbiprofen (17.6)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, glucuronide

KEY WORDS

plasma

REFERENCE

Sallustio, B.C.; Fairchild, B.A. Biosynthesis, characterization and direct high-performance liquid chromatographic analysis of gemfibrozil 1-O- β -acylglucuronide. *J.Chromatogr.B*, **1995**, 665, 345-353

SAMPLE

Matrix: blood

Sample preparation: 0.5 mL Plasma + 0.5 mL 10 μ g/mL IS in MeCN:pH 7.4 phosphate buffered saline 1:99 + 20 μ L formic acid + 5 mL cyclohexane:ethyl acetate 8:2, extract. Remove the organic layer and evaporate it, reconstitute the residue with 500 μ L mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 C₆H₅-1252N (Senshu Sci.)

Mobile phase: MeCN:10 mM pH 3.3 tartrate buffer:PIC-A 52:48:0.5

Flow rate: 1

Injection volume: 10

Detector: F ex 293 em 325

CHROMATOGRAM

Retention time: 7.5

Internal standard: 4-(2,5-dimethylphenoxy)-2,2-dimethylbutanoic acid

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

plasma

REFERENCE

Nakagawa, A.; Shigeta, A.; Iwabuchi, H.; Horiguchi, M.; Nakamura, K.; Takahagi, H. Simultaneous determination of gemfibrozil and its metabolites in plasma and urine by a fully automated high performance liquid chromatographic system. *Biomed. Chromatogr.*, **1991**, *5*, 68–73

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 2 mL MeCN:glacial acetic acid 75:10, vortex thoroughly, centrifuge. Remove the supernatant and add it to 500 mg NaCl, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 5 mm i.d. μ Bondapak C18 radial compression

Mobile phase: MeOH:water:glacial acetic acid 75:24:1

Flow rate: 0.8-1.2

Injection volume: 20

Detector: UV 276

CHROMATOGRAM

Retention time: 9-10

Limit of quantitation: 1 μ g/mL

KEY WORDS

serum

REFERENCE

Forland, S.C.; Chaplin, L.; Cutler, R.E. Assay of gemfibrozil in plasma by "high-performance" liquid chromatography. *Clin. Chem.*, **1987**, *33*, 1938

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 20 μ L 100 μ g/mL ibuprofen in MeCN:water 50:50, acidify with 3 drops 1 M HCl, add 5 mL cyclohexane, shake mechanically for 20 min, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 50-200 μ L mobile phase, inject a 10-20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.6 5 μ m Spherisorb ODS II

Mobile phase: MeCN:water:phosphoric acid 50:50:0.2

Flow rate: 2

Injection volume: 10-20

Detector: UV 225

CHROMATOGRAM

Retention time: 8.8

Internal standard: ibuprofen (5.8)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Hengy, H.; Kollé, E.U. Determination of gemfibrozil in plasma by high performance liquid chromatography. *Arzneimittelforschung*, **1985**, *35*, 1637-1639

SAMPLE

Matrix: blood, tissue

Sample preparation: Acidify plasma with 10 μ L 5 M orthophosphoric acid. Homogenize 1 g tissue with 2 mL ice-cold 10 mM pH 5.0 phosphate buffer containing 1.15% KCl. 1 mL Plasma or tissue homogenate + 5 mL MeCN:acetic acid 96:4, centrifuge at 1000 g for 5 min, discard the supernatant, wash the pellet with 5 mL diethyl ether, wash nine times with 5 mL 4% acetic acid in MeCN: 10 mM pH 5.0 phosphate buffer 2:1. Add 1 mL 1 M KOH and 50 μ L 5 μ g/mL flurbiprofen in 0.06% MeCN to the pellet, heat at 80° overnight, cool, add 500 μ L 4 M HCl, add 5 mL diethyl ether, shake horizontally at 80 oscillations/min for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.5 μ m LiChrosphere 60 C8 RP-select B

Mobile phase: MeCN: water: acetic acid 51:48.5:0.5

Flow rate: 1

Injection volume: 50

Detector: F ex 284 em 316

CHROMATOGRAM

Retention time: 9

Internal standard: flurbiprofen (5)

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, glucuronides

KEY WORDS

rat; plasma; liver; kidney; heart; only gemfibrozil bound to protein is determined by this method

REFERENCE

Sallustio, B.C.; Foster, D.J.R. Reactivity of gemfibrozil 1-O- β -acyl glucuronide. Pharmacokinetics of covalently bound gemfibrozil-protein adducts in rats. *Drug Metab. Dispos.*, **1995**, *23*, 892-899

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma + 250 μ L 200 μ g/mL IS in MeCN: water 5:95 + 1 mL 1 M HCl + 10 mL diethyl ether, shake on a reciprocating shaker for 15 min, centrifuge at 700 g. Remove the organic layer and evaporate it to dryness under a stream of air at 55°, reconstitute the residue in 500 μ L mobile phase, mix, inject a 40 μ L aliquot. (To hydrolyze conjugates mix 1 mL plasma or urine with 25 μ L glucuronidase/sulfatase (Glu-

sulase, DuPont), 1 mL water, and 1 mL 2 M pH 5.2 acetate buffer, heat at 37° overnight, add 2 mL 1 M HCl, proceed as above.)

HPLC VARIABLES

Column: 100 × 4.6 5 μm Partisil ODS-3 RAC II
Mobile phase: MeCN:7 mM phosphoric acid 45:55
Flow rate: 2
Injection volume: 40
Detector: UV 276

CHROMATOGRAM

Retention time: 10.4
Internal standard: 2,2'-dimethyl-5-(2,6-xylyloxy)valeric acid (7.4)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; also for whole blood

REFERENCE

Randinitis, E.J.; Parker, T.D.; Kinkel, A.W. Liquid chromatographic determination of gemfibrozil and its metabolite in plasma. *J.Chromatogr.*, **1986**, *383*, 444-448

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 200 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 300 × 4 5 μm Suplecosil LC-18
Mobile phase: MeOH:water:glacial acetic acid 80:20:1
Flow rate: 0.8
Injection volume: 10
Detector: UV 276

CHROMATOGRAM

Retention time: 14
Internal standard: 2,5-xyleneol (5)

REFERENCE

Supelco Chromatography Products, Supelco, Inc., Bellefonte PA, 1996, p. 155

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10-100 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Supelcosil LC-18-DB or 100 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeOH:water:acetic acid 80:19:1
Flow rate: 1
Injection volume: 10-100
Detector: UV 276

REFERENCE

Luner, P.E.; Babu, S.R.; Radebaugh, G.W. The effects of bile salts and lipids on the physicochemical behavior of gemfibrozil. *Pharm.Res.*, **1994**, *11*, 1755–1760

SAMPLE

Matrix: urine

Sample preparation: 0.5 mL Urine + 0.5 mL 10 µg/mL IS in MeCN, vortex, centrifuge at 2000 g, inject a 10 µL aliquot of the upper layer.

HPLC VARIABLES

Column: 150 × 4.6 YMC-A312 ODS (Yamamura Chemicals)

Mobile phase: MeCN:10 mM pH 4.7 acetate buffer 45:55 for 10.5 min then 80:20

Flow rate: 1 for 10.5 min then 2 mL/min

Injection volume: 10

Detector: F ex 283 em 315 (for gemfibrozil) and ex 300 em 340 (for metabolites)

CHROMATOGRAM

Retention time: 16

Internal standard: 7-(2,5-dimethylphenoxy)-2,2-dimethylheptanoic acid

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

REFERENCE

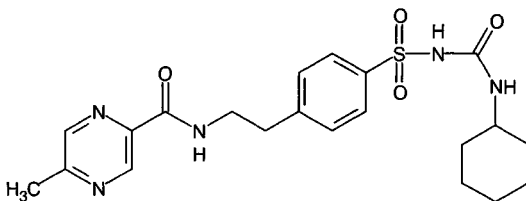
Nakagawa, A.; Shigeta, A.; Iwabuchi, H.; Horiguchi, M.; Nakamura, K.; Takahagi, H. Simultaneous determination of gemfibrozil and its metabolites in plasma and urine by a fully automated high performance liquid chromatographic system. *Biomed.Chromatogr.*, **1991**, *5*, 68–73

Glipizide

Molecular formula: C₂₁H₂₇N₅O₄S

Molecular weight: 445.5

CAS Registry No.: 29094-61-9



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 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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: 3.86

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, doxulepine, doxepin, doxylamine, droperidol, etodolac, fenfluramine, fenpropfen, fentiazac, flecainide, flocetafenine, flumazenil, fluoxetine, fluphenazine, flurbiprofen, flvoxamine, glibenclamide, glibornuride, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozone,

pindolol, pipamperone, piroxicam, prazepam, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, toloxatone, trazodone, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: acebutolol, chlorpropamide, clonazepam, clonidine, ephedrine, estazolam, flunitrazepam, glipizide, glutethimide, melphalan, metoclopramide, prazosin, strychnine, tolbutamide, triazolam

KEY WORDS

whole blood; plasma

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 μ L Plasma + 200 μ L 2 M HCl + 2 mL diethyl ether, vortex for 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 100 μ L mobile phase, vortex for 30 s, inject a 25-50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.1 10 μ m Versapak C18 (Alltech)

Mobile phase: MeCN:10 mM orthophosphoric acid 50:50

Flow rate: 1

Injection volume: 25-50

Detector: UV 230

CHROMATOGRAM

Retention time: 6.49

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, gliclazide, tolazamide, tolbutamide

Simultaneous: N-acetylsulfamethoxazole, sulfamethoxazole

Noninterfering: trimethoprim

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: 2 mL Serum + 2 mL water + 200 μ L 1 (?) M HCl + 200 μ L 2.5 μ g/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 μ L 2 mg/mL dinitrofluorobenzene in butyl ace-

tate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 μ L mobile phase, inject a 120 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 6.5

Internal standard: glibornuride (5.8)

Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, tolazamide, tolbutamide

Interfering: glyburide (glibenclamide) (forms same derivative)

KEY WORDS

serum; derivatization

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 μ L Serum + 800 ng glibornuride + 1 mL 50 mM HCl + 3 mL benzene (Caution! Benzene is a carcinogen!), shake gently for 10 min, centrifuge. Remove organic phase and evaporate it to dryness at 45° under a stream of air. Dissolve residue in 50 μ L MeOH and inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:10 mM pH 3.5 phosphate buffer 60:40

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 9

Internal standard: glibornuride (18)

Limit of detection: 10 ng/mL

KEY WORDS

serum

REFERENCE

Wählin-Boll, E.; Melander, A. High-performance liquid chromatographic determination of glipizide and some other sulfonylurea drugs in serum. *J.Chromatogr.*, **1979**, *164*, 541–546

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μ L Plasma or urine + 300 μ L 10 μ g/mL tolbutamide in MeOH + 1 mL 50 mM HCl + 3 mL benzene (Caution! Benzene is a carcinogen!), shake gently for 15 min, centrifuge at 3250 g for 5 min. Remove organic layer and evaporate it to dryness under a stream of air. Dissolve residue in 50 μ L mobile phase, vortex, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 5 μ m Spherisorb ODS C18

Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 35:65

Flow rate: 1.5

Injection volume: 20

Detector: UV 275

CHROMATOGRAM

Retention time: 10.5

Internal standard: tolbutamide (8.2)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma

REFERENCE

Emilsson, H. High-performance liquid chromatographic determination of glipizide in human plasma and urine. *J.Chromatogr.*, **1987**, *421*, 319–326

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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, dipyron, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fen-

proporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyrl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, pyrildrine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelenamine, 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: whole blood

Sample preparation: 1 mL Whole blood + 1 mL 50 mM pH 6.6 KH_2PO_4 , vortex, add 6 mL diethyl ether, shake at 150 ± 20 oscillations/min on a reciprocating shaker for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness at 37° under a stream of nitrogen. Dissolve residue in 200 μL mobile phase, centrifuge at 1000 g for 3 min, inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150×4.6 5 μm Nucleosil C18

Mobile phase: MeCN:isopropanol:buffer 30:5:65 (Buffer was 80 mM ammonium acetate adjusted to pH 3.5 with concentrated HCl.)

Flow rate: 1

Injection volume: 40

Detector: UV 241

CHROMATOGRAM

Retention time: 5.9

Internal standard: glipizide

OTHER SUBSTANCES

Simultaneous: indapamide

Noninterfering: acetaminophen, aspirin, caffeine, dextromethorphan, ibuprofen, nicotine, phenylpropanolamine, theophylline

KEY WORDS

glipizide is IS

REFERENCE

Miller, R.B.; Dadgar, D.; Lalande, M. High-performance liquid chromatographic method for the determination of indapamide in human whole blood. *J.Chromatogr.*, **1993**, 614, 293–298

ANNOTATED BIBLIOGRAPHY

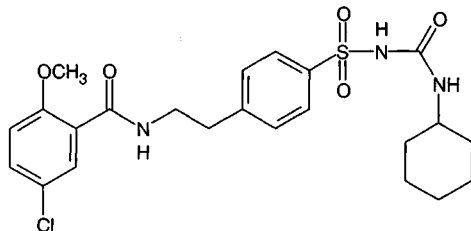
Das Gupta, V. Quantitation of glipizide and glyburide in tablets using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1986**, 9, 3607–3615 [simultaneous glyburide; tablets; hydrocortisone (IS)]

Glyburide

Molecular formula: C₂₃H₂₈ClN₃O₅S

Molecular weight: 494.0

CAS Registry No.: 10238-21-8



SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma + 15 μ L 5 μ g/mL warfarin + 3 mL dichloromethane, vortex for 1 min, shake on a rotary mixer for 5 min, centrifuge at 1000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L mobile phase, inject an 80 μ L aliquot.

HPLC VARIABLES

Guard column: Novapak C18

Column: 150 \times 3.9 4 μ m Novapak C18

Mobile phase: MeOH:50 mM (NH₄)H₂PO₄ 61:39, pH adjusted to 4.0

Flow rate: 1.2

Injection volume: 80

Detector: F ex 308 em 360

CHROMATOGRAM

Retention time: 12.3

Internal standard: warfarin (6.7)

Limit of detection: 20 ng/mL

KEY WORDS

plasma; rat; human; pharmacokinetics

REFERENCE

al-Dhawailie, A.A.; Abdulaziz, M.A.; Tekle, A.; Matar, K.M. A simple, specific, and rapid high-performance liquid chromatographic assay for glibenclamide in plasma. *J.Liq.Chromatogr.*, **1995**, *18*, 3981-3990

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 200 μ L 1 M HCl + 1 μ g tolbutamide + 5 mL toluene, shake gently for 15 min, centrifuge at 1500 g for 3 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 25 μ L 6 mg/mL dinitrofluorobenzene in n-butyl acetate (prepare fresh each week, store at 4° in the dark), heat at 120° for 30 min, evaporate to dryness, reconstitute with 50 μ L mobile phase, inject a 25-50 μ L aliquot. Alternatively, filter (Amicon YMT membrane, 30000 MW cutoff) 200 μ L 100 mM NaOH while centrifuging at 4°, rinse filter with 500 μ L water, filter 1 mL serum in the same unit while centrifuging at 4° at 2500 g for 1.5 h. Remove a 700 μ L aliquot of the ultrafiltrate, add 200 μ L 1 M HCl, add 1 μ g tolbutamide, add 5 mL toluene, shake gently for 15 min, centrifuge at 1500 g for 3 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 25 μ L 6 mg/mL dinitrofluorobenzene in n-butyl acetate (prepare fresh each week, store at 4° in the dark), heat at 120° for 30 min, evaporate to dryness, reconstitute with 50 μ L mobile phase, inject a 25-50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 7 μ m LiChrosorb RP18

Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 80:20

Flow rate: 1

Injection volume: 25-50

Detector: UV 360

CHROMATOGRAM

Retention time: 7

Internal standard: tolbutamide (5)

Limit of detection: 2 ng/mL

KEY WORDS

derivatization; serum; ultrafiltrate; pharmacokinetics

REFERENCE

Arcelloni, C.; Fermo, I.; Calderara, A.; Pacchioni, M.; Pontiroli, A.E.; Paroni, R. Glibenclamide and tolbutamide in human serum: Rapid measurement of the free fraction. *J.Liq.Chromatogr.*, **1990**, *13*, 175-189

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 200 μ L 2 M HCl + 2 mL diethyl ether, vortex for 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 100 μ L mobile phase, vortex for 30 s, inject a 25-50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.1 10 μ m Versapak C18 (Alltech)

Mobile phase: MeCN:10 mM orthophosphoric acid 50:50

Flow rate: 1

Injection volume: 25-50

Detector: UV 230

CHROMATOGRAM

Retention time: 18.52

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, gliclazide, glipizide, tolazamide, tolbutamide

Simultaneous: N-acetylsulfamethoxazole, sulfamethoxazole

Noninterfering: trimethoprim

KEY WORDS

plasma

REFERENCE

Shenfield, G.M.; Boutagy, J.S.; Webb, C. A screening test for detecting sulfonyleureas in plasma. *Ther.Drug Monit.*, **1990**, *12*, 393-397

SAMPLE

Matrix: blood

Sample preparation: 2 mL Serum + 2 mL water + 200 μ L 1 (?) M HCl + 200 μ L 2.5 μ g/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 μ L 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 μ L mobile phase, inject a 120 μ L 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: 6.5
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, tolazamide, tolbutamide
Interfering: glipizide (forms same derivative)

KEY WORDS

serum; derivatization

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: 1 mL Plasma + 100 μL 500 ng/mL IS in MeOH:water 1:1 + 2 mL 100 mM KH₂PO₄ adjusted to pH 4.0 with 1 M orthophosphoric acid, shake for 10 s, add dichloromethane, extract in Rollamix for 30 min, centrifuge at 2000 rpm for 5 min. Remove organic phase and evaporate it to dryness under a stream of air at 50°. Reconstitute residue in 100 μL mobile phase, vortex 30 s, inject 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb C-18
Mobile phase: MeCN:50 mM ammonium sulfate adjusted to pH 3.0 with 1 M sulfuric acid 42:58
Flow rate: 1.4
Injection volume: 50
Detector: UV 229

CHROMATOGRAM

Retention time: 17.8
Internal standard: N-(4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfonyl)-N'-cyclopentylurea (11.4)
Limit of detection: 5 ng/mL

KEY WORDS

plasma

REFERENCE

Othman, S.; Shaheen, O.; Jalal, I.; Awidi, A.; Al-Turk, W. Liquid chromatographic determination of glibenclamide in human plasma. *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 942–944

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 ng tolbutamide + 500 μ L 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 μ L 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 μ L mobile phase, inject a 30-70 μ L aliquot. (Recrystallize dinitrofluorobenzene from diethyl ether. Prepare solutions weekly, store at 4° in the dark.)

HPLC VARIABLES

Column: 125 \times 4.6 5 μ m 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: 3.4
Internal standard: tolbutamide (4.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide
Noninterfering: acetaminophen, aspirin, chlordiazepoxide, diazepam, phenobarbital, phenytoin, quinidine, theophylline

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, urine
Sample preparation: Dilute serum 5-fold with water before analysis. 1 mL Serum or diluted urine + 25 μ L 15 μ g/mL glibornuride in MeOH + 100 μ L 2 M HCl + 6 mL n-hexane: dichloromethane 1:1, rotate for 10 min, centrifuge at 700 g for 10 min. Remove 5 mL of the organic phase and evaporate it to dryness at 37° under a stream of air. Dissolve residue in 50 μ L mobile phase, inject 10-25 μ L aliquots. (If necessary to remove interferences, the organic layer can be washed with 5 mL 25 mM tetrabutylammonium hydrogen sulfate in 50 mM pH 12.2 phosphate buffer by rotation for 10 min.)

HPLC VARIABLES

Guard column: Chrompack reversed-phase
Column: 100 \times 4.6 3 μ m Chrompack Chromsep microsphere C18
Mobile phase: MeCN:38 mM pH 7.49 phosphate buffer 28:72
Flow rate: 0.7
Injection volume: 10-25
Detector: UV 203

CHROMATOGRAM

Retention time: 12.7
Internal standard: glibornuride (6)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Rydberg, T.; Wåhlin-Boll, E.; Melander, A. Determination of glibenclamide and its two major metabolites in human serum and urine by column liquid chromatography. *J.Chromatogr.*, **1991**, *564*, 223–233

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μ L Plasma or urine + 1 mL 50 mM HCl + 25 μ L 10 μ g/mL glibornuride in MeOH + 3 mL benzene (Caution! Benzene is a carcinogen!), shake gently for 15 min, centrifuge at 3250 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air. Dissolve the residue in 50 μ L mobile phase with vortexing, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb ODS

Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 50:50

Flow rate: 1.6

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 8

Internal standard: glibornuride (5.8)

Limit of detection: 5-10 ng/mL

OTHER SUBSTANCES

Noninterfering: chlorpropamide, flunitrazepam, furosemide, glipizide, naproxen, sulfamethoxazole, theophylline, thioridazine, tolbutamide, trimethoprim

KEY WORDS

plasma

REFERENCE

Emilsson, H.; Sjöberg, S.; Svedner, M.; Christenson, I. High-performance liquid chromatographic determination of glibenclamide in human plasma and urine. *J.Chromatogr.*, **1986**, *383*, 93–102

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 2 mg/mL solution in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m Waters phenyl

Mobile phase: MeOH:MeCN:50 mM (NH₄)₂PO₄ 50:10:40

Flow rate: 1.5

Injection volume: 10

Detector: UV 229

CHROMATOGRAM

Retention time: 9.5

Limit of quantitation: 0.02%

OTHER SUBSTANCES**Simultaneous:** impurities

REFERENCE

Beaulieu, N.; Graham, S.J.; Lovering, E.G. Liquid chromatographic determination of glyburide (glibenclamide) and its related compounds in raw materials. *J.AOAC Int.*, **1993**, *76*, 962-965

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:** 8.51 (A), 9.83 (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, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, mocllobemide, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluopromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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

ANNOTATED BIBLIOGRAPHY

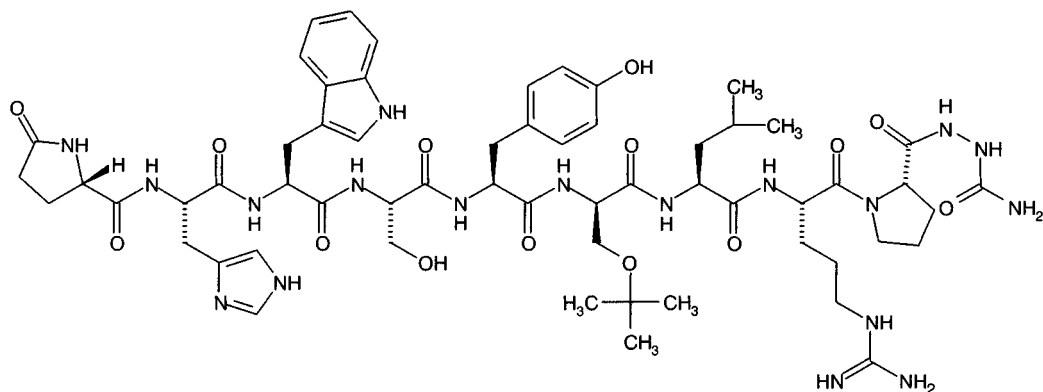
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- Abdel-Hamid, M.E.; Suleiman, M.S.; El-Sayed, Y.M.; Najib, N.M.; Hasan, M.M. A rapid high-performance liquid chromatography assay of glibenclamide in serum. *J.Clin.Pharm.Ther.*, **1989**, *14*, 181–188
- Gupta, R.N. Determination of glyburide in human plasma by liquid chromatography with fluorescence detection. *J.Liq.Chromatogr.*, **1989**, *12*, 1741–1758 [plasma; fluorescence detection; SPE; chlorowarfarin (IS)]
- Das Gupta, V. Quantitation of glipizide and glyburide in tablets using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1986**, *9*, 3607–3615 [simultaneous glipizide; tablets; hydrocortisone (IS)]
- Potter, H.; Hulm, M. [Determination of glibenclamide in blood using high performance liquid chromatography]. *J.Chromatogr.*, **1983**, *273*, 217–222
- Adams, W.J.; Skinner, G.S.; Bombardt, P.A.; Courtney, M.; Brewer, J.E. Determination of glyburide in human serum by liquid chromatography with fluorescence detection. *Anal.Chem.*, **1982**, *54*, 1287–1291
- Uihlein, M.; Sistovaris, N. High-performance liquid column and thin-layer chromatographic determination of human serum glibenclamide at therapeutic levels. *J.Chromatogr.*, **1982**, *227*, 93–101

Goserelin

Molecular formula: C₅₉H₈₄N₁₈O₁₄

Molecular weight: 1269.4

CAS Registry No.: 65807-02-5



SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 200 × 3 Spherisorb S5ODS-2

Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% (NH₄)₂SO₄. 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 76:24

Flow rate: 0.5

Detector: UV 210

CHROMATOGRAM

Retention time: 14.5 (gradient), 11 (isocratic)

OTHER SUBSTANCES

Simultaneous: buserelin, deslorelin, gonadorelin, leuprolide, nafarelin

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

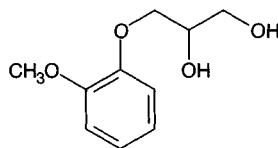
Corran, P.H.; Sutcliffe, N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography. *J.Chromatogr.*, **1993**, *636*, 87-94

Guaifenesin

Molecular formula: C₁₀H₁₄O₄

Molecular weight: 198.2

CAS Registry No.: 93-14-1



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 2.58 μ g/mL laudanosine in MeCN + 500 μ L saturated sodium carbonate solution, vortex for 10 s, add 5 mL chloroform, vortex for 10 s, mix on a rocking mixer for 40 min, centrifuge at 2000 g for 25 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μ L mobile phase, inject a 300 μ L aliquot. (Hydrolyze conjugates by heating 1 mL plasma with 1 mL 3000 U/mL β -glucuronidase (*Helix pomatia* type H-1 (Sigma)) in 100 mM pH 5.0 sodium citrate at 37° for 2 h, proceed as above.)

HPLC VARIABLES

Column: 150 \times 4.6 Spherisorb 5-CN

Mobile phase: MeCN:water:triethylamine 10:89:1 adjusted to pH 6 with orthophosphoric acid

Flow rate: 1

Injection volume: 300

Detector: F ex 280 em 315

CHROMATOGRAM

Retention time: 1.275

Internal standard: laudanosine (8.603)

Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Extracted: levorphanol (dextrorphan)

KEY WORDS

plasma

REFERENCE

Stavchansky, S.; Demirbas, S.; Reyderman, L.; Chai, C.-K. Simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 919–925

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma + 100 μ L 200 μ g/mL mephenesin in water, mix, add 5 mL ethyl acetate, shake for 15 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen. Reconstitute in 500 μ L of water, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Microsorb C18

Mobile phase: MeOH:100 mM KH₂PO₄:water 35:10:55

Column temperature: 40

Flow rate: 0.8

Injection volume: 50

Detector: UV 272

CHROMATOGRAM**Retention time:** 4.8**Internal standard:** mephenesin (10.5)

OTHER SUBSTANCES**Simultaneous:** methocarbamol**Noninterfering:** acetaminophen, ibuprofen

KEY WORDS

plasma

REFERENCE

Naidong, W.; Lee, J.W.; Hulse, J.D. Development and validation of a high-performance liquid chromatographic method for the determination of methocarbamol in human plasma. *J.Chromatogr.B*, **1994**, *654*, 287–292

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Plasma + 100 μ L 2.5 mg/mL O-desmethylnaproxen in MeOH + 400 μ L acetone, homogenize for 10 min, centrifuge at 1000 g for 15 min. Remove supernatant and evaporate it to dryness under a stream of air at 35°. Take up residue in 500 μ L mobile phase, inject 10 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m LiChrosorb RP-18**Mobile phase:** MeOH:10 mM pH 6.5 citrate buffer 10:90**Column temperature:** 35**Flow rate:** 2**Injection volume:** 10**Detector:** F ex 230 em 306

CHROMATOGRAM**Retention time:** 37**Internal standard:** O-desmethylnaproxen (26)

OTHER SUBSTANCES**Simultaneous:** metabolites

KEY WORDS

plasma; horse

REFERENCE

Ketelaars, H.C.; Peters, J.G.; Anzion, R.B.; Van Ginneken, C.A. Isolation, partial identification and quantitative determination of four guaifenesin glucuronides in plasma and urine of the horse by high-performance liquid chromatography. *J.Chromatogr.*, **1984**, *288*, 423–429

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Plasma + 50 μ L 1 mg/mL mephenesin in water + 60 μ L 1 M HCl, homogenize, add 5 mL diethyl ether, shake for 30 min, centrifuge at 1000 g for 15 min. Remove ether layer and evaporate it to dryness at 30° under a stream of air. Take up residue in 500 μ L mobile phase and inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m LiChrosorb RP-8

Mobile phase: MeOH:10 mM pH 6.5 citrate buffer 40:60

Column temperature: 30

Flow rate: 1

Detector: UV 275

CHROMATOGRAM

Retention time: 2.8

Internal standard: mephenesin (8.8)

OTHER SUBSTANCES

Simultaneous: β -(2-methoxyphenoxy)lactic acid

KEY WORDS

plasma

REFERENCE

Ketelaars, H.C.J.; Peters, J.G.P. Determination of guaifenesin and its metabolite, beta-(2-methoxyphenoxy)-lactic acid, in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1981**, *224*, 144-148

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 μ m Millipore), inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 4.5 μ m 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 284

CHROMATOGRAM

Retention time: 5.2

Limit of detection: 2.8 μ g/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen (UV 310), caffeine (UV 298), chlorpheniramine (UV 265), phenylpropanolamine (UV 260)

KEY WORDS

tablets

REFERENCE

Indrayanto, G.; Sunarto, A.; Adriani, Y. Simultaneous assay of phenylpropanolamine hydrochloride, caffeine, paracetamol, glycerylguaiacolate and chlorpheniramine in SilabatTM tablet using HPLC with diode array detection. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1555-1559

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:** 3.35

OTHER SUBSTANCES**Simultaneous:** benzoic acid, dextromethorphan

KEY WORDS

liquid formulation; 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:** 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:** 3.7

OTHER SUBSTANCES**Simultaneous:** benzoic acid, dextromethorphan, saccharin

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:** 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:** 7.5

OTHER SUBSTANCES**Simultaneous:** impurities, degradation products, benzoic acid, phenylephrine, phenylpropanolamine

KEY WORDStablets; 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**Sample preparation:** Dilute with water, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 300 \times 4 μm Bondapak C18**Mobile phase:** MeOH:water:glacial acetic acid 45:55:2 containing 5 mM octanesulfonic acid**Flow rate:** 2.5**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 2.2

OTHER SUBSTANCES**Simultaneous:** acetaminophen, butyl paraben, ethyl paraben, methyl paraben, pholcodine, propyl paraben, pseudoephedrine

KEY WORDScough mixture

REFERENCE

Carnevale, L. Simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture by high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, *72*, 196–198

SAMPLE**Matrix:** formulations**Sample preparation:** Capsules and Tablets. Leach 1 g of ground capsule or tablet with 250 mL 0.4 mg/mL 2,5-dihydroxybenzoic acid in water, sonicate for 10 min, centrifuge at

2500 rpm for 5 min, inject an aliquot. Liquid formulations. Dilute 4-25 mL of the formulation to 250 mL with 0.4 mg/mL 2,5-dihydroxybenzoic acid in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 C8

Mobile phase: MeOH:water:PIC-B5 300:675:25 (PIC-B5 (Waters) is 200 mM sodium pentanesulfonate in glacial acetic acid.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Internal standard: 2,5-dihydroxybenzoic acid (4.5)

OTHER SUBSTANCES

Simultaneous: impurities, degradation products, phenylephrine, phenylpropanolamine

KEY WORDS

capsules; tablets; liquid formulations; stability-indicating

REFERENCE

Schieffer, G.W.; Hughes, D.E. Simultaneous stability-indicating determination of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and guaifenesin in dosage forms by reversed-phase paired-ion high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, *72*, 55-59

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 (Prepare ammonium formate buffer by diluting 34 mL 28-30% ammonia with 30 mL water, add 30 mL 98% formic acid (Caution! Exothermic!), cool, dilute this mixture (pH 3.9) to 100 mL with water.)

Flow rate: 2

Injection volume: 15

Detector: UV 280

CHROMATOGRAM

Retention time: 6

Internal standard: o-dinitrobenzene (11)

OTHER SUBSTANCES

Simultaneous: acetaminophen, p-aminophenol, dextromethorphan

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: 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: 4.94 (A), 3.93 (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, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, 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, methyl-dopa, 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, phenolamine, 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, sulfonpyrazone, 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

some 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: 450 μL Buffer solution + 50 μL 4 mg/mL acetanilide, cool in ice, inject a 10 μL aliquot.

HPLC VARIABLES

Column: Perkin-Elmer 3 \times 3 CR C-18

Mobile phase: MeCN:water 10:90 containing 1% acetic acid

Flow rate: 2.5

Injection volume: 10

Detector: UV 274

CHROMATOGRAM

Retention time: 3

Internal standard: acetanilide (2.1)

OTHER SUBSTANCES

Simultaneous: methocarbamol

KEY WORDS

buffers

REFERENCE

Pouli, N.; Antoniadou-Vyzas, A.; Foscolos, G.B. Methocarbamol degradation in aqueous solution. *J.Pharm.Sci.*, **1994**, 83, 499–501

SAMPLE

Matrix: solutions

Sample preparation: Dissolve compounds in MeCN:water 80:20, inject a 1 μL aliquot.

HPLC VARIABLES

Column: 150 \times 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: 8

OTHER SUBSTANCES

Simultaneous: acetaminophen, buccetin (3-hydroxy-p-butyrophenetidine), caffeine, dipyrrone (sulpyrin), methyl p-hydroxybenzoate, phenacetin

KEY WORDS

semi-micro

REFERENCE

Matsushima, Y.; Nagata, Y.; Niyomura, M.; Takakusagi, K.; Takai, N. Analysis of antipyretics by semi-micro liquid chromatography. *J.Chromatogr.*, **1985**, 332, 269–273

ANNOTATED BIBLIOGRAPHY

Alvi, S.U.; Castro, F. A simultaneous assay of theophylline, ephedrine hydrochloride, and phenobarbital in suspensions and tablets formulations by high performance liquid chromatography. *J.Liq. Chromatogr.*, **1986**, 9, 2269–2279 [simultaneous ephedrine, phenobarbital, theophylline; suspensions; tablets; guaifenesin is IS]

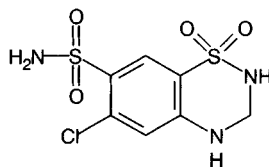
Muhammad, N.; Bodnar, J.A. Quantitative determination of guaifenesin, phenylpropanolamine hydrochloride, sodium benzoate & codeine phosphate in cough syrups by high-pressure liquid chromatography. *J.Liq.Chromatogr.*, **1980**, *3*, 113–122 [simultaneous benzoic acid, codeine, phenylpropanolamine; syrup]

Hydrochlorothiazide

Molecular formula: C₇H₈ClN₃O₄S₂

Molecular weight: 297.7

CAS Registry No.: 58-93-5



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 20 μ g/mL hydroflumethiazide in MeOH + 1 mL buffer + 200 μ L water + 6 mL ethyl acetate, shake for 5 min, centrifuge at 900 g for 5 min. Remove 5 mL organic layer and evaporate at 37 $^{\circ}$ under a stream of nitrogen. Reconstitute with 100 μ L MeOH, sonicate twice at 37 $^{\circ}$ for 1 min, cool at 2-8 $^{\circ}$ for 2 h to obtain a clear solution, inject a 20 μ L aliquot. (Buffer was 0.38 g ammonium acetate in 500 mL water, acidified to pH 5.0 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 40 \times 4 35-50 μ m C18 Corasil

Column: 125 \times 4 5 μ m Nucleosil 100-5 C18

Mobile phase: Gradient. A was MeCN:acetic acid:water 25:1:975. B was MeCN:acetic acid:water 500:1:500. A:B from 100:0 to 36:64 over 16 min, re-equilibrate at 100:0 for 24 min before next injection

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 9.0

Internal standard: hydroflumethiazide (12.0)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: acebutolol, acenocoumarol, acetaminophen, aspirin, allopurinol, ambroxol, amoxicillin, atenolol, bendroflumethiazide, benzbromarone, bezafibrate, biperiden, bisacodyl, bromazepam, butizide, caffeine, captopril, cimetidine, ciprofloxacin, clobutinol, clonidine, cotinine, diazepam, diclofenac, digitoxin, digoxin, dihydrocodeine, dihydroergotamine, diltiazem, doxepin, doxycycline, enalapril, erythromycin, fenoterol, furosemide, glibenclamide, heparin, hypoxanthine, ibuprofen, indomethacin, isosorbide mononitrate, lisinopril, lovastatin, maprotiline, methyldigoxin, methyldopa, metoclopramide, metoprolol, metronidazole, midazolam, naloxone, nifedipine, nicotine, norfloxacin, ofloxacin, oxazepam, oxipurinol, penicillin V, pentoxifylline, phenacetin, phenazone, propyphenazone, phenprocoumon, ranitidine, salicylic acid, sotalol, sulfamethoxazole, trimethoprim, terbutaline, theophylline, tilidine, timolol, triamterene, uric acid, verapamil, vitamin C, warfarin, xanthine, purine and pyrimidine bases, nucleosides, nucleotides

KEY WORDS

plasma; amiloride interferes with IS

REFERENCE

de Vries, J.X.; Voss, A. Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography. *Biomed. Chromatogr.*, **1993**, *7*, 12-14

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 25 μ L 20 μ g/mL procainamide.HCl in MeOH, vortex, add 5 mL MTBE, place on a reciprocating shaker at low speed for 15 min, centrifuge at 1250 g for 10 min. Remove organic layer and evaporate under a stream of nitrogen. Reconstitute in 200 μ L mobile phase, inject 100 μ L aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 Supelco Pelliguard C18

Column: 150 × 4.6 3 μm Hypersil ODS

Mobile phase: MeCN:7 mM sodium heptanesulfonate 18:82, containing 1% glacial acetic acid and 0.035% triethylamine

Flow rate: 0.8

Injection volume: 100

Detector: UV 272

CHROMATOGRAM

Retention time: 5.1

Internal standard: procainamide.HCl (10.1)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, aspirin, ibuprofen

KEY WORDS

plasma

REFERENCE

Azumaya, C.T. Sensitive liquid chromatographic method for the determination of hydrochlorothiazide in human plasma. *J.Chromatogr.*, **1990**, 532, 168–174

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: UV 271

CHROMATOGRAM

Retention time: 4.80

Internal standard: hydroflumethiazide (7.94)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amiloride (detection by F)

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, urine

Sample preparation: Filter (0.45 μm) urine or plasma. Mix plasma filtrate with an equal volume of 50 mM pH 8.0 Tris-sulfuric acid buffer containing 0.1 mM zinc acetate and 40 mM sodium dodecyl sulfate. Inject a 50 μL aliquot of the urine filtrate or the diluted plasma onto column A with mobile phase A, elute to waste with mobile phase A, after 5 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. Re-equilibrate column A with mobile phase A for 5 min before next injection.

HPLC VARIABLES

Column: A 10 \times 4.6 carbonic anhydrase (Prepare by adding 3 g aminopropyl silica from a Sep-Pak NH₂ SPE cartridge to 30 mL 100 mg/mL N, N'-disuccinimidyl carbonate in MeCN in portions over 30 min with gentle mixing, mix for 3 h, filter (G-5 glass), wash the solid 5 times with 50 mL portions of MeCN. Add 100 mg activated gel to 2 mL 200 mM pH 8.0 phosphate buffer containing 1 M NaCl, degas by sonicating under aspirator vacuum, add 2 mL 2.5 mg/mL carbonic anhydrase in water, shake at room temperature for 4 h, centrifuge, discard the supernatant, suspend the gel in 50 mM pH 8.0 Tris-sulfuric acid buffer, slurry pack into column. Store in 50 mM pH 8.0 Tris-sulfuric acid buffer containing 0.1 mM zinc acetate when not in use.); B 150 \times 4.6 Cosmosil 5C18-AR (Nakarai Tesque)

Mobile phase: A 50 mM pH 8.0 Tris-sulfuric acid buffer containing 0.1 mM zinc acetate; B MeCN:100 mM pH 5.2 acetate buffer 10:90 containing 500 mM NaCl

Flow rate: 1

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 7.5

Limit of detection: 100 nM

Limit of quantitation: 1 μM

OTHER SUBSTANCES

Also analyzed: acetazolamide, chlorothiazide, chlorthalidone, furosemide

Noninterfering: acetaminophen, bumetanide, caffeine, phenylbutazone, salicylic acid, sulfamerazine, sulfamethiazole, sulfamethoxazole, sulfamonomethoxine, sulfisomidine, sulfisoxazole, theophylline, tolbutamide, warfarin

KEY WORDS

plasma; column-switching

REFERENCE

Ohta, T.; Takamiya, I.; Takitani, S. Carbonic anhydrase-immobilized precolumn for selective on-line sample pretreatment in high-performance liquid chromatographic determination of certain sulphonamide drugs. *Biomed.Chromatogr.*, **1994**, *8*, 184-188

SAMPLE

Matrix: feed

Sample preparation: 2 g Feed + 20 mL MeCN, rotate at 20 rpm for 1 h, centrifuge at 1300 rpm for 15 min, inject an aliquot.

HPLC VARIABLES

Guard column: 100 \times 6.3 30-38 μm Co:Pell ODS (Whatman)

Column: 250 \times 4.6 10 μm Lichrosorb RP-2

Mobile phase: MeOH:water: 5:95 (flush column with MeCN:water 50:50 after use)

Flow rate: 2

Injection volume: 50

Detector: UV 265

CHROMATOGRAM

Retention time: 7

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: formulations

Sample preparation: Dilute 1000-fold, mix a 200 μL aliquot with 200 μL 100 $\mu\text{g}/\text{mL}$ hydrochlorothiazide, inject a 20 μL aliquot.

HPLC VARIABLES

Column: Microsorb MV C-18

Mobile phase: MeCN:water:acetic acid 6:93:1, adjusted to pH 4.0 with 6 M NaOH

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Internal standard: hydrochlorothiazide

OTHER SUBSTANCES

Simultaneous: ceftazidime

KEY WORDS

injections; saline; stability-indicating; hydrochlorothiazide is IS

REFERENCE

Bednar, D.A.; Klutman, N.E.; Henry, D.W.; Fox, J.L.; Strayer, A.H. Stability of ceftazidime (with arginine) in an elastomeric infusion device. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1912–1914

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 \times 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: 6.7

Internal standard: caffeine (4.8)

OTHER SUBSTANCES

Simultaneous: enalapril

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: 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 Na₂HPO₄, pH adjusted to 3.0 with HCl.)

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 1.6

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: carbidopa, dopamine, epinephrine, 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 5 fold with mobile phase. Mix the diluted formulation with an equal volume of 50 µg/mL hydrochlorothiazide, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:0.1% phosphoric acid 55:45

Flow rate: 1

Injection volume: 10

Detector: UV 260

CHROMATOGRAM

Retention time: 5.6

Internal standard: hydrochlorothiazide

OTHER SUBSTANCES

Simultaneous: captopril

KEY WORDS

syrup; hydrochlorothiazide is IS

REFERENCE

Nahata, M.C.; Morosco, R.S.; Hipple, T.F. Stability of captopril in three liquid dosage forms. *Am.J.Hosp.Pharm.*, **1994**, *51*, 95-96

SAMPLE

Matrix: formulations

Sample preparation: Crush tablets, weigh out amount equivalent to one tablet, add 40 mL MeOH, warm on a steam bath for 5 min, cool to room temperature, make up to 100 mL with MeOH, filter through paper, inject 100 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Porasil

Mobile phase: MeOH

Flow rate: 1.5

Injection volume: 100

Detector: F ex 280 em 360; UV 360

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: reserpine (by F only)

KEY WORDS

tablets; normal phase

REFERENCE

Cieri, U.R. Determination of reserpine and hydrochlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorption detectors in series. *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 515-518

SAMPLE

Matrix: formulations

Sample preparation: Grind tablets, add 3-20 mL MeCN:water 15:85, sonicate for 10 min, filter, make up to 100 mL with MeCN:water 15:85. Remove a 500 μ L aliquot and add it to 300 μ L 250 μ g/mL procaine hydrochloride in water, make up to 10 mL with mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m ASI chromosphere 3869 octadecylsilane (Analytical Sciences, Inc.)

Mobile phase: MeCN:50 mM NaH₂PO₄ 30:70 containing sodium pentanesulfonate, pH adjusted to 2.5 with concentrated phosphoric acid

Flow rate: 1

Injection volume: 50

Detector: E, Metrohm model E-611, Bioanalytical Systems Kel F cell, glassy carbon electrode +1300 mV, auxiliary platinum electrode, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.5

Internal standard: procaine hydrochloride (5.7)

Limit of quantitation: 1.25 μ g/mL

OTHER SUBSTANCES**Simultaneous:** guanethidine

KEY WORDS

tablets; not stability-indicating

REFERENCEStewart, J.T.; Clark, S.S. Liquid chromatographic determination of guanethidine salts and hydrochlorothiazide using electrochemical detection and ion-pair techniques. *J.Pharm.Sci.*, **1986**, 75, 413-415

SAMPLE**Matrix:** formulations**Sample preparation:** Injections. Dilute 1.5 mL of a 20 mg/mL injection to 100 mL with water, remove a 10 mL aliquot and add it to 3 mL 0.2% hydrochlorothiazide, make up to 100 mL with water, inject a 20 μ L aliquot. Tablets. Grind tablets to a fine powder, weigh out amount equivalent to about 10 mg hydralazine, mix thoroughly with 2 mL 500 mM HCl, make up to 100 mL with water, shake for 2-3 min, filter, discard first 15 mL. 15 mL Filtrate + 1.5 mL 0.2% hydrochlorothiazide, make up to 50 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 μ Bondapak phenyl**Mobile phase:** MeOH:15 mM KH_2PO_4 :glacial acetic acid 0.5:99.4:0.1**Flow rate:** 3**Injection volume:** 20**Detector:** UV 256

CHROMATOGRAM**Retention time:** 8**Internal standard:** hydrochlorothiazide

OTHER SUBSTANCES**Simultaneous:** hydralazine, phenylpropanolamine

KEY WORDS

injections; tablets; hydrochlorothiazide is IS

REFERENCEDas Gupta, V. Quantitation of hydralazine hydrochloride in pharmaceutical dosage forms using high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1985**, 8, 2497-2509

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 4.6 10 μ m Partisil ODS1**Mobile phase:** MeOH:50 mM pH 3.0 phosphoric acid 10:90**Column temperature:** 30**Flow rate:** 1.5**Detector:** Radioactivity

OTHER SUBSTANCES**Also analyzed:** atenolol, cimetidine, ranitidine

KEY WORDS¹⁴C labeled

REFERENCE

Collett, A.; Sims, E.; Walker, D.; He, Y.-L.; Ayrton, J.; Rowland, M.; Warhurst, G. Comparison of HT29-18-C₁ and Caco-2 cell lines as models for studying intestinal paracellular drug absorption. *Pharm.Res.*, **1996**, *13*, 216-221

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), 3.98 (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, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, 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, methdiazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methylolpam, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thienthylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 μ L aliquot.

HPLC VARIABLES

Guard column: 5 \times 4 10 μ m LiChrosorb RP-8

Column: 100 \times 4.6 5 μ m 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 \times 0.3 mm coil of PTFE tubing irradiated at 254 nm with a Sylvania GTE 8 W low-pressure lamp to the detector.

CHROMATOGRAM

Limit of detection: 133 ng/mL

OTHER SUBSTANCES

Also analyzed: bendroflumethiazide, butizide, chlorthalidone, ethacrynic acid, furosemide

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

Guard column: 30 \times 3.2 7 μ m SI 100 ODS (not commercially available)

Column: 150 \times 3.2 7 μ m 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 211, 268

CHROMATOGRAM

Retention time: 1.6

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, 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 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, cortisolone, 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, fenpropofen, 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, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, 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, pyrillamine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triproilidine, 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 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 10:1.5:0.5:88

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 2.64

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 × 2 C18 glass lined (Whatman)

Mobile phase: MeCN:water 60:40

Flow rate: 0.04

Injection volume: 0.5

Detector: UV 254; MS, Hewlett Packard 5985, home-made interface (details in paper)

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Simultaneous: chlorothiazide, 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:** urine**Sample preparation:** Inject an aliquot 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, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES**Column:** A 30 μm 20 \times 2.1 Hypersil ODS-C18; B 250 \times 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:** 6.3**Limit of detection:** 7 ng/mL

OTHER SUBSTANCES**Simultaneous:** acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, furosemide, probenecid, spironolactone, triamterene

REFERENCECampíns-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

SAMPLE**Matrix:** urine**Sample preparation:** 1 mL Urine + 100 μL 20 $\mu\text{g/mL}$ hydroflumethiazide in MeOH + 1 mL buffer + 200 μL water + 6 mL ethyl acetate, shake for 5 min, centrifuge at 900 g for 5 min. Remove 5 mL organic layer and evaporate at 37° under a stream of nitrogen. Reconstitute with 100 μL mobile phase, inject a 20 μL aliquot. (Buffer was 0.38 g ammonium acetate in 500 mL water, acidified to pH 5.0 with glacial acetic acid.)

HPLC VARIABLES**Guard column:** 40 \times 4 35-50 μm C18 Corasil**Column:** 125 \times 4 5 μm Nucleosil 100-5 C18**Mobile phase:** MeCN:acetic acid:water 120:1:880**Flow rate:** 1**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 5.0**Internal standard:** hydroflumethiazide (10.0)**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Noninterfering:** amiloride, acebutolol, acenocoumarol, acetaminophen, aspirin, allopurinol, ambroxol, amoxicillin, atenolol, bendroflumethiazide, benzbromarone, bezafibrate, biperiden, bisacodyl, bromazepam, butizide, captopril, cimetidine, ciprofloxacin, clobutinol, clonidine, cotinine, diazepam, diclofenac, digitoxin, digoxin, dihydrocodeine, dihydroergotamine, diltiazem, doxepin, doxycycline, enalapril, erythromycin, fenoterol, furosemide, glibenclamide, heparin, hypoxanthine, ibuprofen, indomethacin, isosorbide

mononitrate, lisinopril, lovastatin, maprotiline, methyl digoxin, methyl dopa, metoclopramide, metoprolol, metronidazole, midazolam, naloxone, nifedipine, nicotine, oxazepam, oxipurinol, penicillin V, pentoxifylline, phenacetin, phenazone, propyphenazone, phenprocoumon, ranitidine, salicylic acid, sotalol, sulfamethoxazole, trimethoprim, terbutaline, theophylline, tilidine, timolol, triamterene, uric acid, verapamil, vitamin C, warfarin, xanthine, purine and pyrimidine bases, nucleosides, nucleotides

Interfering: caffeine

KEY WORDS

norfloxacin and ofloxacin interfere with IS

REFERENCE

de Vries, J.X.; Voss, A. Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography. *Biomed.Chromatogr.*, **1993**, *7*, 12–14

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 and elute to waste 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 \times 4.5 μm Hypersil octadecylsilica ODS; B 200 \times 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 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 270

CHROMATOGRAM

Retention time: 11.3

Limit of detection: 1 $\mu\text{g}/\text{mL}$

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, 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 $\mu\text{g}/\text{mL}$ 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: 3.0

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene, 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: 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; UV 270

CHROMATOGRAM

Retention time: 4.3

Limit of detection: 150 ng (by MS)

OTHER SUBSTANCES

Extracted: acetazolamide

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 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 μ L 50 μ g/mL β -hydroxyethyltheophylline in MeOH, inject 5 μ L aliquot. (Solid buffer I was $\text{KH}_2\text{PO}_4:\text{Na}_2\text{HPO}_4$ 99:1, solid buffer II was $\text{NaHCO}_3:\text{K}_2\text{CO}_3$ 3:2.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 6.03 (A), 7.07 (B)

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

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benthiazide, bumetanide, canrenone, chlorothiazide, chloretalidone, cyclothiazide, dichlorphenamide, ethacrynic acid, flumethiazide, furosemide, hydroflumethiazide, methyclothiazide, metolazone, polythiazide, probenecid, quinethazone, spironolactone, triamterene, trichloromethiazide

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: 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 μ L MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4 5 μ m 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: 2.2

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: bendroflumethiazide, bumetanide, chlorothiazide, chlorthalidone, clopamide, cyclopenthiiazide, furosemide, mefruside, methyclothiazide, metolazone, quinethazone

Simultaneous: clorexolone, ethacrynic acid, indapamide

Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyl dopa, 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

ANNOTATED BIBLIOGRAPHY

Cieri, U.R. Determination of reserpine, hydralazine HCl, and hydrochlorothiazide in tablets by liquid chromatography on a short, normal-phase column. *J.AOAC Int.*, **1994**, *77*, 1104–1108 [simultaneous hydralazine, reserpine; tablets; normal-phase; fluorescence detection; UV detection]

Hsieh, J.Y.-K.; Lin, C.; Matuszewski, B.K.; Dobrinska, M.R. Fully automated methods for the determination of hydrochlorothiazide in human plasma and urine. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1555–1562 [plasma; urine; SPE; LOQ 2 ng/mL]

Ulvi, V.; Keski-Hynnälä, H. First-derivative UV spectrophotometric and high-performance liquid chromatographic analysis of some thiazide diuretics in the presence of their photodecomposition products. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 917–922 [simultaneous degradation products; also chlorothiazide, trichlormethiazide]

Abdelhameed, M.H.; Chen, T.M.; Chi, W.L. Intrahepatic distribution of hydrochlorothiazide and quinidine in rats: Implications in pharmacokinetics. *J.Pharm.Sci.*, **1993**, *82*, 992–996 [whole blood; plasma; liver; chlorothiazide (IS); rat; pharmacokinetics]

Berthod, A.; Laserna, J.J.; Carretero, ... Oil-in-water microemulsions as mobile phases for rapid screening of illegal drugs in sports. *J.Liq.Chromatogr.*, **1992**, *15*, 3115–3127 [simultaneous acebutolol, chlorthalidone, codeine, hydrochlorothiazide, methoxamine, methyltestosterone, nadolol, norcodeine, oxprenolol, phenylephrine, probenecid]

Chen, T.M.; Abdelhameed, M.H.; Chiou, W.L. Erythrocytes as a total barrier for renal excretion of hydrochlorothiazide: slow influx and efflux across erythrocyte membranes. *J.Pharm.Sci.*, **1992**, *81*, 212–218 [whole blood; plasma; chlorothiazide (IS); human; rat; pharmacokinetics]

Herráez-Hernández, R.; Campíns-Falcó, P.; Sevillano-Cabeza, A. Improved screening procedure for diuretics. *J.Liq.Chromatogr.*, **1992**, *15*, 2205–2224 [LOD 10–1000 ng/mL; gradient; urine; hydroxymethyltheophylline (IS); extracted acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, furosemide, probenecid, spironolactone, triamterene]

Miller, R.B.; Amestoy, C. A liquid chromatographic method for the determination of hydrochlorothiazide in human plasma. *J.Pharm.Biomed.Anal.*, **1992**, *10*, 541–545

Campíns-Falcó, P.; Herráez-Hernández, R.; Sevillano-Cabeza, A. Solid-phase extraction techniques for assay of diuretics in human urine samples. *J.Liq.Chromatogr.*, **1991**, *14*, 3575–3590 [urine; SPE; hydroxyethyltheophylline (IS); extracted acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, furosemide, probenecid, spironolactone, triamterene]

Bachman, W.J.; Stewart, J.T. HPLC-photolysis-electrochemical detection in pharmaceutical analysis: application to the determination of spironolactone and hydrochlorothiazide in tablets. *J.Chromatogr.Sci.*, **1990**, *28*, 123–128 [post-column reaction; electrochemical detection; tablets; simultaneous spironolactone]

Kuo, B.S.; Mandagere, A.; Osborne, D.R.; Hwang, K.K. Column-switching high-performance liquid chromatographic (HPLC) determination of hydrochlorothiazide in rat, dog, and human plasma. *Pharm.Res.*, **1990**, *7*, 1257–1261

- Santasia, C.T. Direct injection analysis of diuretic and anti-inflammatory drugs on a shielded hydrophobic phase column. *J.Liq.Chromatogr.*, **1990**, *13*, 2605–2631 [serum; direct injection; gradient; horse; extracted furosemide, oxyphenbutazone, phenylbutazone]
- Sa'sa', S.I.; Jalal, I.M.; Khalil, H.S. Determination of atenolol combinations with hydrochlorothiazide and chlorthalidone in tablet formulations by reverse-phase HPLC. *J.Liq.Chromatogr.*, **1988**, *11*, 1673–1696 [simultaneous atenolol, chlorthalidone; tablets; methyl p-hydroxybenzoate (IS); stability-indicating]
- Hitscherich, M.E.; Rydberg, E.M.; Tsilifonis, D.C.; Daly, R.E. Simultaneous determination of hydrochlorothiazide and propranolol hydrochloride in tablets by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1987**, *10*, 1011–1021 [simultaneous propranolol; tablets; stability indicating]
- Valkó, K. RP-HPLC retention data for measuring structural similarity of compounds for QSAR studies. *J.Liq.Chromatogr.*, **1987**, *10*, 1663–1686 [also acetanilide, acetylazidomorphine, aspirin, azidocodaine, azidoethylmorphine, azidomorphine, barbital, benzaldehyde, benzoic acid, bromocyanonitrophenol, caffeine, chloramphenicol, chlorocyanonitrophenol, chloronitroaniline, cortexolone, cortisone, cyanodinitrophenol, cyanofluoronitrophenol, cyclopropylazidoethylmorphine, cyclopropylmethylazidomorphine, 11-deoxycorticosterone, dexamethasone, dichloronitroaniline, dinitroaniline, dinitrophenol, hydrocortisone, isoniazid, methyl salicylate, morphine, niacinamide, nicotinamide, nitroaniline, nitrophenol, norazidoethylmorphine, norazidomorphine, normorphine, phenacetin, phenobarbital, phenylethylazidoethylmorphine, phenylethylazidomorphine, prednisolone, progesterone, salicylamide, salicylic acid, sulfadimidine, sulfaguanidine, sulfamethazine, sulfamethoxy-pyridazine, testosterone, triamcinolone, trinitroaniline, trinitrophenol, vanillin, vitamin B3, vitamin B5]
- Alton, K.B.; Desrivieres, D.; Patrick, J.E. High-performance liquid chromatographic assay for hydrochlorothiazide in human urine. *J.Chromatogr.*, **1986**, *374*, 103–110
- Shiu, G.K.; Prasad, V.K.; Lin, J.; Worsley, W. Simple and selective high-performance liquid chromatographic method for the determination of hydrochlorothiazide in urine. *J.Chromatogr.*, **1986**, *377*, 430–435
- Kirschbaum, J.; Perlman, S. Analysis of captopril and hydrochlorothiazide combination tablet formulations by liquid chromatography. *J.Pharm.Sci.*, **1984**, *73*, 686–687
- Koopmans, P.P.; Tan, Y.; Van Ginneken, C.A.; Gribnau, F.W. High-performance liquid chromatographic determination of hydrochlorothiazide in plasma and urine. *J.Chromatogr.*, **1984**, *307*, 445–450
- Yamazaki, M.; Ito, Y.; Suzuka, T.; Yaginuma, H.; Itoh, S.; Kamada, A.; Orita, Y.; Nakahama, H.; Nakanishi, T.; Ando, A. Biopharmaceutical studies of thiazide diuretics. II. High-performance liquid chromatographic method for determination of hydrochlorothiazide in plasma, urine, blood cells and bile. *Chem.Pharm.Bull.*, **1984**, *32*, 2387–2394
- Shah, V.P.; Walker, M.A.; Prasad, V.K. Application of flow programming in the analysis of drugs and their metabolites in biological fluids. *J.Liq.Chromatogr.*, **1983**, *6*, 1949–1954 [extracted metabolites, chlorothiazide, triamterene; flow programming; urine; plasma]
- Barbhaiya, R.H.; Phillips, T.A.; Welling, P.G. High-pressure liquid chromatographic determination of chlorothiazide and hydrochlorothiazide in plasma and urine: preliminary results of clinical studies. *J.Pharm.Sci.*, **1981**, *70*, 291–295
- Daniels, S.L.; Vanderwielen, A.J. Stability-indicating assay for hydrochlorothiazide. *J.Pharm.Sci.*, **1981**, *70*, 211–215
- Menon, G.N.; White, L.B. Simultaneous determination of hydrochlorothiazide and triamterene in capsule formulations by high-performance liquid chromatography. *J.Pharm.Sci.*, **1981**, *70*, 1083–1085 [capsules; m-hydroxyacetophenone (IS); simultaneous triamterene]
- Henion, J.D.; Maylin, G.A. Qualitative and quantitative analysis of hydrochlorothiazide in equine plasma and urine by high-performance liquid chromatography. *J.Anal.Toxicol.*, **1980**, *4*, 185–191

Hydrocodone

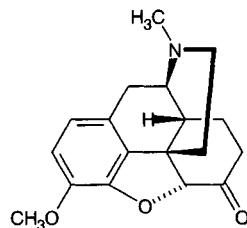
Molecular formula: C₁₈H₂₁NO₃

Molecular weight: 299.4

CAS Registry No.: 125-29-1 (hydrocodone),

34195-34-1 (hydrocodone bitartrate

hydrate), 143-71-5 (hydrocodone bitartrate)



SAMPLE

Matrix: bile, blood

Sample preparation: 0.5 mL Blood or bile + 10 (blood) or 15 (bile) μ L 100 μ g/mL nalorphine in MeOH + 300 μ L 1.1 M pH 5.0 sodium acetate buffer + 3000-3500 U of *Patella vulgata* glucuronidase, incubate at 55° overnight, add 0.5 mL borate buffer to achieve a pH of 8.3-8.5. Add 8 mL chloroform:isopropanol 90:10, gently rotate for 30 min, centrifuge at 3500 rpm for 10 min, remove aqueous layer. Wash organic layer (twice for blood, three times for bile) with 3 mL 100 mM pH 9.9 sodium phosphate buffer with gentle rotation for 10 min and centrifugation each time. Add organic layer to 200 (blood) or 400 (bile) μ L 0.2% phosphoric acid, gently rotate for 30 min, discard organic layer, inject 50 μ L of the acid layer. (Borate buffer was 50 mM boric acid and 43 mM sodium tetraborate, adjusted to pH 9.8.)

HPLC VARIABLES

Guard column: Nova-Pak phenyl guard column

Column: 150 \times 3.9 5 μ m Nova-Pak phenyl

Mobile phase: MeCN:10 mM pH 6.6 NaH₂PO₄ 10:90

Flow rate: 1.2

Injection volume: 50

Detector: UV 210; F ex 220 em 370 (cut-off)

CHROMATOGRAM

Retention time: 38.1

Internal standard: nalorphine (23.5)

OTHER SUBSTANCES

Simultaneous: codeine, dihydrocodeine, 6-monoacetylmorphine, morphine, oxycodone

Noninterfering: acetylcodeine, amitriptyline, amphetamine, diamorphine, diazepam, dothiepin, doxepin, ephedrine, ephedrine, hydromorphone, mesoridazine, methadone, methamphetamine, 3-monoacetylmorphine, nordiazepam, norpropoxyphene, nortriptyline, oxazepam, propoxyphene, pseudoephedrine, quinidine, quinine, sulfamethoxazole, sulfonidazine, thioridazine

KEY WORDS

UV and F detection used together

REFERENCE

Crump, K.L.; McIntyre, I.M.; Drummer, O.H. Simultaneous determination of morphine and codeine in blood and bile using dual ultraviolet and fluorescence high-performance liquid chromatography. *J. Anal. Toxicol.*, 1994, 18, 208-212

SAMPLE

Matrix: formulations

Sample preparation: Measure out syrup equivalent to about 5 mg hydrocodone bitartrate, add 5 mL water, add 1 mL 1.8 M sulfuric acid, wash twice with 40 mL portions of chloroform. Make the aqueous layer alkaline with 5 mL 1 M NaOH, extract twice with 25

mL portions of chloroform. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 500 μ L EtOH, inject the whole amount.

HPLC VARIABLES

Column: 305 \times 7 PRP-1 (Hamilton)

Mobile phase: Gradient. A was water:triethylamine 99.9:0.1. B was MeCN:triethylamine 99.9:0.1. A:B 60:40 for 7 min, to 20:80 over 5 min, maintain at 20:80 for 5 min, to 60:40 over 6 min, re-equilibrate at 60:40 for 2 min.

Column temperature: 40

Flow rate: 3.5

Injection volume: 500

Detector: UV 254

CHROMATOGRAM

Retention time: 6.9

OTHER SUBSTANCES

Simultaneous: diphenylpyraline, doxylamine, etafedrine, guaifenesin, pheniramine, phenylephrine, phenylpropanolamine, pyrilamine

KEY WORDS

syrup

REFERENCE

Black, D.B.; By, A.W.; Lodge, B.A. Isolation and identification of hydrocodone in narcotic cough syrups by high-performance liquid chromatography with infrared spectrometric identification. *J.Chromatogr.*, **1986**, 358, 438-443

SAMPLE

Matrix: formulations

Sample preparation: Add 1 tablet to 95 mL water, place on a steam bath for 15 min, cool, mix for 15 min, sonicate, allow to stand, filter, inject 13 μ L aliquot

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeOH:buffer 25:75 (Buffer was 0.01 N KH_2PO_4 + 50 mM KNO_3 , adjusted to pH 4.5 with 3 N phosphoric acid.)

Flow rate: 1.1

Injection volume: 13

Detector: UV 283

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: acetaminophen, p-aminophenol, p-chloroacetanilide, codeine, hydromorphone

KEY WORDS

tablets; stability-indicating

REFERENCE

Wallo, W.E.; D'Adamo, A. Simultaneous assay of hydrocodone bitartrate and acetaminophen in a tablet formulation. *J.Pharm.Sci.*, **1982**, 71, 1115-1118

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: 6.93 (A), 3.71 (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, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, 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, methyl-dopa, 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, sulfonpyrazone, 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

some 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 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, dapson, 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, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaacol, halazepam, haloperidol, hydrochlorothiazide, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenthylin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscipine, nyldrin, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triaminolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triproilidine, 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 MeOH at a concentration of 1 mg/mL, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 4.18

OTHER SUBSTANCES

Simultaneous: acetylcodeine, amphetamine, benzphetamine, benzylmorphine, bromo-STP, buprenorphine, caffeine, chlorphentermine, codeine, codeine-N-oxide, dextromoramide, dextropropoxyphene, diamorphine, diethylpropion, dihydrocodeine, dihydromorphine, dimethylamphetamine, dipipanone, ephedrine, epinephrine, ethoheptazine, ethylmorphine, etorphine, fencamfamin, fenethyline, fenfluramine, fentanyl, 4-hydroxyamphetamine, hydroxypethidine, levallorphan, levorphanol, mazindol, meperidine, mephentermine, methadone, methylenedioxymphetamine, methylephedrine, methylphenidate, monoacetylmorphine, morphine, morphine-3-glucuronide, morphine-N-oxide, nalorphine, naloxone, norcodeine, norlevorphanol, normetanephrine, normethadone, normorphine, norpipanone, norpseudoephedrine, noscapine, oxycodone, papaverine, pemoline, pentazocine, phenazocine, phendimetrazine, phenelzine, 2-phenethylamine, phenoperidine, phentermine, phenylephrine, phenylpropanolamine, pholcodeine, pipradol, piritramide, prolintane, pseudoephedrine, STP, thebacon, thebaine, tranlylcypromine, trimethoxyamphetamine, tyramine

Noninterfering: dopamine, levodopa, methyl dopa, methyl dopate, norepinephrine

Interfering: mescaline, methamphetamine, norpethidine

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: 500 μ L Urine + N-ethylordiazepam + chlorpheniramine + 100 μ L buffer, centrifuge at 11000 g for 30 s, inject a 500 μ L 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 μ L mobile phase B, with 200 μ L 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 KH₂PO₄ 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 KH₂PO₄ 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 KH₂PO₄ 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 KH₂PO₄ containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: 40 (B, C only)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210; UV 235

CHROMATOGRAM

Retention time: k' 8.0

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, amphetamine, benzoylecgonine, caffeine, codeine, cotinine, desipramine, diazepam, diphenhydramine, ephedrine, flurazepam, hydromorphone, imipramine, lidocaine, methadone, methamphetamine, morphine, nordiazepam, nortriptyline, oxazepam, pentazocine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, 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

ANNOTATED BIBLIOGRAPHY

Alvi, S.U.; Castro, F. A stability-indicating simultaneous analysis of acetaminophen and hydrocodone bitartrate in tablets formulation by HPLC. *J.Liq.Chromatogr.*, **1987**, *10*, 3413–3426 [stability-indicating; simultaneous impurities, acetaminophen, codeine, hydromorphone; tablets; column temp 30]

Fatmi, A.A.; Williams, G.V. Simultaneous determination of acetaminophen and hydrocodone bitartrate in solid dosage forms by HPLC. *J.Liq.Chromatogr.*, **1987**, *10*, 2461–2472

Hydrocortisone

Molecular formula: C₂₁H₃₀O₅

Molecular weight: 362.5

CAS Registry No.: 50-23-7, 13609-67-1 (butyrate), 57524-89-7

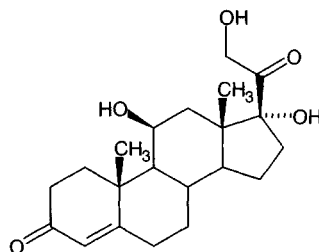
(valerate), 50-03-3 (acetate), 3863-59-0 (phosphate),

6000-74-4 (sodium phosphate), 125-04-2

(21-sodium succinate), 508-96-3 (tebutate), 74050-20-7

(aceponate), 72590-77-3 (buteprate), 508-99-6 (cypionate),

83784-20-7 (hemisuccinate monohydrate), 2203-97-6 (hemisuccinate)



SAMPLE

Matrix: amniotic fluid, blood

Sample preparation: Centrifuge serum or amniotic fluid for 10 min. 0.5-1 mL Serum or amniotic fluid + 500 μ L MeOH:water 5:95, mix, inject 750 μ L 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 \times 4.6 5 μ m Medipola-ODS C18 (Sekisui)

Mobile phase: A water; B MeCN:MeOH:buffer 2:7:20 (Buffer was 6.8 g/L KH₂PO₄, 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: 66

Limit of detection: 7.8 ng

OTHER SUBSTANCES

Extracted: cortisone, estetrol, estriol

Noninterfering: androstenedione, corticosterone, hydroxyprogesterone, progesterone, testosterone

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: 750 μ L Serum + 75 μ L MeOH + 100 μ L 1.5 μ g/mL dexamethasone in 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 μ L ethyl acetate, inject a 17 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 4 5 μ m 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: 12.79

Internal standard: dexamethasone (11.43)

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: prednisolone, prednisolone acetate

KEY WORDS

serum; normal phase

REFERENCE

Döppenschmitt, 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: 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 onto column A and elute to waste with mobile phase, after 3 min divert the effluent from column A onto 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: 23

OTHER SUBSTANCES

Extracted: cortisone, 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 (3M Co.) by adding 500 μ L MeOH and forcing through three drops, discard the remaining liquid, add water, force through three drops, discard the water. 300 μ L Serum + 150 μ L IS solution, let stand at room temperature for 10 min, add 800 μ L saturated sodium borate solution, mix, centrifuge at 12400 g for 3 min (if necessary), add to the extraction disc, centrifuge at 100-120 g for 5 min, force through 200 μ L water, force through 500 μ L MeOH:water 18:82, elute with 50 μ L MeCN then 150 μ L water, mix the eluates, inject a 20 μ L 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 \times 2 30 μ m Permaphase ETH (Du Pont)**Column:** 250 \times 2 Ultrasphere C18 or 250 \times 4.6 Ultrasphere C18**Mobile phase:** THF:water 20:80 (Use a 150 \times 4.6 37-53 μ m silica gel (Whatman) saturating column (held at 55°) between the pump and the injector.)**Column temperature:** 55**Flow rate:** 0.18 (250 \times 2) or 0.8 (250 \times 4.6)**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 13**Internal standard:** fludrocortisone (15), methylprednisolone (20)**Limit of detection:** 4 ng/mL

OTHER SUBSTANCES**Extracted:** corticosterone, cortisone, prednisolone, prednisone**Simultaneous:** aldosterone, androsteindione, beclomethasone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisone, dexamethasone, 17-hydroxyprogesterone, metyrapone, pregnenolone, progesterone, testosterone, triamcinolone

KEY WORDSserum; SPE; extraction disc

REFERENCELensmeyer, 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:** Extract 1 mL plasma containing dexamethasone with 12 mL dichloromethane. Remove the organic phase and wash it with 2 mL 100 mM NaOH, wash with 1 mL water, dry over 1 g anhydrous sodium sulfate. Evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 3 μ m Spherisorb silica**Mobile phase:** Hexane:dichloromethane:EtOH:glacial acetic acid 26:69:3.4:2**Flow rate:** 0.75**Detector:** UV 254

CHROMATOGRAM**Retention time:** 6.3

Internal standard: dexamethasone (5.1)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: methylprednisolone, prednisolone

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Möllmann, H.; Hochhaus, G.; Rohatagi, S.; Barth, J.; Derendorf, H. Pharmacokinetic/pharmacodynamic evaluation of deflazacort in comparison to methylprednisolone and prednisolone. *Pharm.Res.*, **1995**, *12*, 1096–1100

SAMPLE

Matrix: blood

Sample preparation: Extract plasma with 12 mL dichloromethane, wash the organic layer with 2 mL 100 mM NaOH and 1 mL water, dry the organic layer over 1 g anhydrous sodium sulfate. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb silica

Mobile phase: Hexane:dichloromethane:EtOH:glacial acetic acid 26:69:3.4:2

Flow rate: 0.75

Detector: UV 254

CHROMATOGRAM

Retention time: 11.6

Internal standard: methylprednisolone (13.4)

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: triamcinolone acetonide

Noninterfering: cortisone

KEY WORDS

plasma; normal phase

REFERENCE

Rohatagi, S.; Hochhaus, G.; Möllmann, J.; Barth, J.; Galia, E.; Erdmann, M.; Sourgens, H.; Derendorf, H. Pharmacokinetic and pharmacodynamic evaluation of triamcinolone acetonide after intravenous, oral, and inhaled administration. *J.Clin.Pharmacol.*, **1995**, *35*, 1187–1193

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 NaH₂PO₄ 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; UV 256; UV 343

CHROMATOGRAM

Retention time: 14.37

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

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone, chloroquine, corticosterone, cortisolone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydroxychloroquine, 17 β -hydroxyprogesterone, meprednisone, methylprednisolone acetate, methylprednisolone, paramethasone, prednisolone, prednisone, progesterone, triamcinolone

KEY WORDS

serum

REFERENCE

Volin, P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, 666, 347-353

SAMPLE

Matrix: blood

Sample preparation: Prepare a Sep-Pak Plus Environmental C18 SPE cartridge by washing with 15 mL MeOH then 15 mL water. 1 mL Serum + 100 μ L 3 μ g/mL betamethasone in isopropanol:MeCN 1:1 + 100 μ L isopropanol:acetonitrile 1:1, mix, add to SPE cartridge, wash with 10 mL water, elute with 3 mL MeOH. Evaporate the eluate at 50 $^{\circ}$ under a stream of nitrogen, reconstitute in 200 μ L mobile phase A, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 guard column

Column: 250 \times 4.6 5 μ m 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: 26

Internal standard: betamethasone (33)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, cortisolone, prednisolone, prednisone

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: 1 mL Plasma + 50 μ L 4 μ g/mL betamethasone in EtOH + 15 mL dichloromethane, shake horizontally for 15 min, centrifuge at 1500 g for 15 min. Remove the organic layer and wash it with 100 μ L 100 mM NaOH then 1 mL water. Remove the aqueous phase and dry the organic phase over 1 g of anhydrous sodium sulfate. Evaporate the organic phase to dryness under a stream of nitrogen at not more than 37°, reconstitute in 200 μ L mobile phase, inject a 175 μ L aliquot.

HPLC VARIABLES**Guard column:** 20 \times 2 30-38 μ m HC Pellosil**Column:** 250 \times 4.6 5-6 μ m Zorbax SIL**Mobile phase:** Heptane:dichloromethane:glacial acetic acid:ethanol 350:600:10:35**Flow rate:** 2**Injection volume:** 175**Detector:** UV 254

CHROMATOGRAM**Retention time:** 15**Internal standard:** betamethasone (12)**Limit of detection:** 5 ng/mL**Limit of quantitation:** 10 ng/mL

OTHER SUBSTANCES**Simultaneous:** prednisolone, prednisone**Noninterfering:** cyclosporin, ethinyl estradiol, ketoconazole, levonorgestrel, rapamycin, tacrolimus, tenidap, tetrahydrocortisone

KEY WORDS

plasma; normal phase

REFERENCE

Jusko, W.J.; Pyszczynski, N.A.; Bushway, M.S.; D'Ambrosio, R.; Mis, S.M. Fifteen years of operation of a high-performance liquid chromatographic assay for prednisolone, cortisol and prednisone in plasma. *J.Chromatogr.B*, **1994**, 658, 47–54

SAMPLE**Matrix:** blood

Sample preparation: 100 μ L 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 μ L mobile phase, inject a 20-80 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m 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: 9

Internal standard: beclomethasone (22)

Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES

Extracted: dexamethasone

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: 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 μ L 400 ng/mL flumethasone in MeOH:water 5:95, heat at 50° for 10 min, add to the 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 μ L mobile phase, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 2.3 μ m 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: 4.5

Internal standard: flumethasone (13)

Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, cortisone

Simultaneous: acebutolol, acetazolamide, acetophenetidin, adrenosterone, aldosterone, amitriptyline, androsten-3,17-dione, aspirin, carbamazepine, cephalothin, chlorothiazide, dehydrocorticosterone, deoxycorticosterone, deoxycortisol, desipramine, dexamethasone, diazepam, 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, quinine, spironolactone, testosterone, theophylline, triamcinolone, tripeleminamine

Noninterfering: allopurinol, caffeine, cotinine, ephedrine, nicotine, phenylephrine

Interfering: chlordiazepoxide, diphenhydramine, propranolol

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 μ L Plasma + 10 μ L 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 μ L MeCN. Add 10 μ L reagent 1, add 10 μ L reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μ L water, add 200 μ L 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 μ L by evaporation at 40° under reduced pressure, inject 20 μ L 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.)

HPLC VARIABLES**Guard column:** 50 \times 4.6 7 μ m Zorbax ODS**Column:** 250 \times 4.6 7 μ m 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:** 26.5**Internal standard:** fluocinolone acetonide (40.7)**Limit of detection:** 0.6-3 pg/mL

OTHER SUBSTANCES**Simultaneous:** aldosterone, corticosterone, cortisone, 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: Condition a Tef Elutor C18 SPE cartridge with two 3 mL portions of MeOH then two 3 mL portions of water. 1 mL Plasma + 50 μ L 400 ng/mL flumethasone in 5:95 MeOH:water, heat at 50° for 10 min, add to the SPE 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 μ L mobile phase, inject 25 μ L 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: 3.98

Internal standard: flumethasone (11.50)

Limit of detection: 300 pg/mL

OTHER SUBSTANCES

Simultaneous: adrenosterone, amitriptyline, aspirin, carbamazepine, corticosterone, cortisolone, deoxycorticosterone, desipramine, dexamethasone, diazepam, diphenhydramine, equilenin, estradiol, estriol, estrone, fluorometholone, hydroxyprogesterone, imipramine, indomethacin, methylprednisolone, nordiazepam, nortriptyline, phenobarbital, prednisolone, prednisone, probenecid, progesterone, propranolol, spironolactone, testosterone, theophylline, tripeleennamine

Noninterfering: acebutolol, acetazolamide, acetophenetidin, aldosterone, allopurinol, caffeine, cephalothin, chlorothiazide, cotinine, ephedrine, furosemide, hydrochlorothiazide, nicotine, pheniramine, phenylephrine, phenytoin, primidone, quinine, triamcinolone

Interfering: chlordiazepoxide

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 Serum + 500 μL water + 100 μL 10 μg/mL 3,7-dimethoxyflavone in EtOH + 8 mL diethyl ether, shake, centrifuge at 4° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:water 40:60, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 μm NS-Gel C18

Mobile phase: Gradient. MeOH:water from 40:60 to 55:45, maintain at 55:45 for 24 min, to 80:20 over 25 min.

Column temperature: 50

Flow rate: 1

Injection volume: 50

Detector: UV 210; UV 240

CHROMATOGRAM

Retention time: 17.00

Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES

Extracted: aldosterone, androstenedione, dehydroepiandrosterone, 11-deoxycortisol, deoxycorticosterone, estradiol, estrone, 17-hydroxyprogesterone, progesterone, pregnenolone

KEY WORDS

serum

REFERENCE

Ueshiba, H.; Segawa, M.; Hayashi, T.; Miyachi, Y.; Irie, M. Serum profiles of steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method. *Clin.Chem.*, **1991**, *37*, 1329-1333

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Plasma + 100 μ L 3 M sulfuric acid + 50 μ L 6 μ g/mL prednisone in MeCN:MeOH 50:50, mix, add 15 mL hexane:ethyl acetate 50:50, shake for 20 min, centrifuge, freeze at -70° . Remove the organic layer and add it to 1 mL 1 M nitric acid, shake, freeze. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 30° , reconstitute the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES**Guard column:** 12.5 \times 4 5 μ m Zorbax SIL**Column:** three 80 \times 4 5 μ m Zorbax SIL Reliance 5 columns in series**Mobile phase:** Dichloromethane:hexane:EtOH:glacial acetic acid 69:26:2.3:1 (Pass the mobile phase through a 70 \times 6 37-53 μ m HC-Pellocil (Whatman) column.)**Flow rate:** 2**Detector:** UV 254

CHROMATOGRAM**Retention time:** 15**Internal standard:** prednisone (9)**Limit of detection:** 15 ng/mL

OTHER SUBSTANCES**Extracted:** methylprednisolone, methylprednisolone hemisuccinate

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kong, A.-N.; Slaughter, R.L.; Jusko, W.J. Simultaneous analysis of methylprednisolone hemisuccinate, cortisol and methylprednisolone by normal-phase high-performance liquid chromatography in human plasma. *J.Chromatogr.*, **1988**, *432*, 308-314

SAMPLE**Matrix:** blood

Sample preparation: 100 μ L Plasma + 200 μ L IS solution, vortex 1 min, centrifuge at 1500 g for 10 min, inject 50 μ L of supernatant. (Prepare IS solution by dissolving 200 μ g n-propyl p-hydroxybenzoate in 10 mL MeOH, add 2 mL glacial acetic acid, dilute 1 mL of this solution with 9 mL MeOH.)

HPLC VARIABLES**Column:** 300 \times 4 μ Bondapak C18**Mobile phase:** MeCN:buffer 23:77 (Buffer was 50 mM sodium acetate + 100 mM NaCl, adjusted to pH 2.8 with acetic acid.)**Flow rate:** 2.5**Injection volume:** 50**Detector:** UV 254

CHROMATOGRAM

Retention time: 4.8 (hydrocortisone), 8.4 (hydrocortisone succinate)

Internal standard: n-propyl p-hydroxybenzoate (7.2)

Limit of detection: 200 ng/mL; 500 ng/mL (succinate)

OTHER SUBSTANCES

Simultaneous: bromhexine, noscapine, tipepidine

Noninterfering: albuterol, orciprenaline, terbutaline, theophylline

KEY WORDS

plasma

REFERENCE

Iwasaki, E. Hydrocortisone succinate and hydrocortisone simultaneously determined in plasma by reversed-phase liquid chromatography, and their pharmacokinetics in asthmatic children. *Clin.Chem.*, **1987**, 33, 1412-1415

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge by washing with 2 mL MeCN, 2 mL acetone:water 2:98, and 4 mL water. Do not allow cartridge to run dry. 2 mL Plasma + 40 μ L 5 μ g/mL dexamethasone in MeOH, add to the 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 μ L dichloromethane, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 27

Internal standard: dexamethasone (23.5)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: cortisone, prednisolone, prednisolone acetate, prednisone

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: 2 mL Plasma + 40 μ L 5 μ g/mL dexamethasone in MeOH, vortex 30 s, add 5 mL dichloromethane:diethyl ether 50:50, vortex for 15 s, repeat extraction, com-

bine 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: 27

Internal standard: dexamethasone (23.5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: cortisone, prednisolone, prednisolone acetate, prednisone

KEY WORDS

plasma; normal phase; pig

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: 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: 10

Internal standard: dexamethasone (8)

Limit of detection: 2 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: beclomethasone, betamethasone, corticosterone, cortisone, fluocinonide, methylprednisolone, methylprednisone, prednisolone, prednisone

KEY WORDS

plasma; normal phase

REFERENCE

Ebling, W.F.; Szefer, 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: 1 mL Plasma + 10 μ L 10 μ g/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 μ L mobile phase and inject.

HPLC VARIABLES

Column: 100 \times 8 radial compression 10 μ m 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: 6.0

Internal standard: prednisolone (7.5)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: corticosterone, dexamethasone

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 + 150 ng dexamethasone + 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak/Corasil (Waters)

Column: 300 \times 3.9 10 μ m μ Porasil (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: 6.5

Internal standard: dexamethasone (5)

OTHER SUBSTANCES

Extracted: prednisolone, prednisone

KEY WORDS

plasma; normal phase

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 + 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

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: 4.5

Internal standard: equilenin (7.5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: betamethasone, deoxycortisol, dexamethasone, prednisone, triamcinolone

Interfering: prednisolone

KEY WORDS

Anal.Abs. 1982, 43, 4D182; 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 + 4 mL 59.5 ng/mL triamcinolone acetonide in dichloromethane, shake at high speed for 15 min, centrifuge at 2000 rpm for 10 min, remove aqueous layer, add 5 mL saturated sodium bicarbonate solution to the organic layer, shake at high speed for 5 min, centrifuge at 2000 rpm for 10 min, remove aqueous layer. Place

organic layer in a pointed tube and evaporate to dryness at 45° under a stream of nitrogen. Reconstitute with 50 µL mobile phase, inject 20 µL aliquot.

HPLC VARIABLES

Column: 10 µm Porasil

Mobile phase: Hexane:dichloromethane:ethanol:acetic acid 68.8:25:6:0.2

Flow rate: 2.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: triamcinolone acetonide (3)

OTHER SUBSTANCES

Simultaneous: prednisolone, prednisone

KEY WORDS

plasma; normal phase

REFERENCE

Agabeyoglu, I.T.; Wagner, J.G.; Kay, D.R. A sensitive high-pressure liquid chromatographic method for the determination of prednisone, prednisolone and hydrocortisone in plasma. *Res. Commun. Chem. Pathol. Pharmacol.*, **1980**, *28*, 163–176

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 µL 2 µg/mL dexamethasone in 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: 14

Internal standard: dexamethasone (11.5)

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, 11-deoxycortisol, 17-hydroxyprogesterone, 6α-methylprednisolone, prednisolone, prednisone, progesterone

KEY WORDS

plasma; normal phase

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, 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 μ L under a stream of nitrogen at 37°, inject a 5-20 μ L 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 μ L MeOH:water 65:35, inject a 5-20 μ L aliquot. Tissue. Homogenize (Polytron) fetal tissue in 10-15 mL MeOH:dimethoxymethane 50:50 for 1 min or until breakup is 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 μ L MeOH:water 65:35, centrifuge, inject a 5-20 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 70 \times 6 35-50 μ m Bondapak C18 Corasil

Column: 250 \times 10 5 μ m 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: 31.07

OTHER SUBSTANCES

Extracted: metabolites, cortexolone, cortisol glucuronide, cortisone, 6 β -hydroxycortisol, triamcinolone, triamcinolone acetonide

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: 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 \times 2.1 10 μ m 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: 2.54 (hydrocortisone-21-acetate)

OTHER SUBSTANCES

Simultaneous: bamipine lactate, beclomethasone dipropionate, betamethasone-17-valerate, dexamethasone

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: Weigh out ointment corresponding to 50-300 µg hydrocortisone, add mobile phase, warm until a fine dispersion formed, make up to 100 mL with mobile phase, filter (0.45 µm), inject a 100 µL aliquot.

HPLC VARIABLES

Column: 250 mm long 5 µm Hypersil ODS

Mobile phase: MeOH:water 65:35 adjusted to pH 3 with 85% phosphoric acid

Flow rate: 1

Injection volume: 100

Detector: UV (wavelength not specified)

CHROMATOGRAM

Limit of detection: 20 ng/mL

KEY WORDS

ointment

REFERENCE

Preiss, A.; Mehnert, W.; Frömmling, K.-H. Penetration of hydrocortisone into excised human skin under the influence of cyclodextrins. *Pharmazie*, **1995**, *50*, 121–126

SAMPLE

Matrix: formulations

Sample preparation: Dissolve tablet in 10 mM HCl containing 90 mM KCl (pH 2.0), inject an aliquot.

HPLC VARIABLES

Column: 50 mm long ODS Hypersil C18

Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 30:70

Flow rate: 1

Detector: UV 257

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES**Simultaneous:** nitrofurantoin

KEY WORDS

tablets

REFERENCE

Neervannan, S.; Dias, L.S.; Southard, M.Z.; Stella, V.J. A convective-diffusion model for dissolution of two non-interacting drug mixtures from co-compressed slabs under laminar hydrodynamic conditions. *Pharm.Res.*, **1994**, *11*, 1288-1295

SAMPLE**Matrix:** formulations

Sample preparation: Ointment. 50 mg Ointment + 10 mL ether, vortex until dissolved. Remove a 200 μ L aliquot and add phenyl salicylate in mobile phase, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 10 mL mobile phase, warm for 1 min on a steam bath, vortex for 1 min, cool. Remove an aliquot, dilute with mobile phase, inject an aliquot. Cream. Suspend 50 mg cream in 10 mL mobile phase by vortexing. Remove an aliquot and add phenyl salicylate in mobile phase, evaporate to dryness under a stream of nitrogen at 40°, suspend the residue in 10 mL mobile phase, warm for 1 min on a steam bath, vortex for 1 min, cool. Remove an aliquot, dilute with mobile phase, inject an aliquot.

HPLC VARIABLES**Guard column:** 40 \times 5 RP-18-MPLC (Brownlee)**Column:** 250 \times 2.6 ODS-HC-SIL-X (Perkin-Elmer)**Mobile phase:** MeOH:50 mM phosphoric acid 70:30 (Flush column with MeOH at the end of each day.)**Column temperature:** 40**Flow rate:** 1**Injection volume:** 20**Detector:** UV 256

CHROMATOGRAM**Retention time:** 3**Internal standard:** phenyl salicylate (5.25)

OTHER SUBSTANCES**Simultaneous:** iodochlorhydroxyquin

KEY WORDS

ointment; cream

REFERENCE

Ezzedeen, F.W.; Stohs, S.J.; Masoud, A.N. High-performance liquid chromatographic analysis of iodochlorhydroxyquin and hydrocortisone in ointments and creams. *J.Pharm.Sci.*, **1983**, *72*, 1036-1039

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 μ L 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 240

CHROMATOGRAM**Retention time:** 2**Internal standard:** bromobenzene (3.5)**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Simultaneous:** clioquinol

KEY WORDSointment

REFERENCE

Phoon, K.W.; Stubbley, 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 a sample equivalent to about 10 mg active ingredient, add 20 mL warm MeOH:water 4:1, shake vigorously, add 20 mL n-hexane, extract. Remove the hexane and extract it twice with 10 mL MeOH:water 4:1. Combine all aqueous layers and make up to 50 mL with MeOH:water 4:1, inject 20 µL aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 µBondapak C18**Mobile phase:** MeOH:water 7:3**Flow rate:** 1**Injection volume:** 20**Detector:** UV 240

CHROMATOGRAM**Retention time:** 6 (hydrocortisone acetate)

KEY WORDSointment; cream

REFERENCE

Lea, A.R.; Kennedy, J.M.; Low, G.K.C. Analysis of hydrocortisone acetate ointments and creams by high-performance liquid chromatography. *J.Chromatogr.*, **1980**, *198*, 41–47

SAMPLE**Matrix:** formulations**Sample preparation:** Ointment. Dissolve 0.5 g ointment in 10 mL chloroform, make up to 25 mL with chloroform, inject 20 µL aliquot. Cream. Heat 0.5 g cream in a vacuum desiccator at 60° for 2-4 h, cool, dissolve residue in 10 mL chloroform, make up to 25 mL with chloroform, inject 20 µL aliquot.

HPLC VARIABLES**Column:** 250 × 2 Varian SI-10

Mobile phase: Cyclohexane:isopropanol 90:10

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 4 (hydrocortisone acetate)

KEY WORDS

ointment; cream; normal phase

REFERENCE

Lea, A.R.; Kennedy, J.M.; Low, G.K.C. Analysis of hydrocortisone acetate ointments and creams by high-performance liquid chromatography. *J.Chromatogr.*, **1980**, *198*, 41–47

SAMPLE

Matrix: formulations, solutions

Sample preparation: Ointment. 1 g Ointment + 5 mL MeOH + 5 mL water + 800 μ L 1 mg/mL hydrocortisone in EtOH, stir until a clear solution forms, make up to 25 mL with water, inject a 20 μ L aliquot. Solutions. 8 mL Solution + 800 μ L 1 mg/mL hydrocortisone in EtOH + 5 mL MeOH, make up to 25 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 μ Bondapak C18

Mobile phase: MeCN:200 mM KH_2PO_4 32:68, pH 4.2

Flow rate: 3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4

Internal standard: hydrocortisone

OTHER SUBSTANCES

Simultaneous: triamcinolone acetonide

KEY WORDS

ointment; hydrocortisone is IS

REFERENCE

Das Gupta, V. Stability of triamcinolone acetonide solutions as determined by high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, *72*, 1453–1456

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 6 α -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 $^\circ$, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 Newguard RP-18

Column: two 250 \times 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: 19

Internal standard: 6 α -methylprednisolone (30)

Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: metabolites, cortisone, dihydrocortisol, dihydrocortisone

Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, carbamazepine, cholesterol, clonazepam, dehydroepiandrosterone, dexamethasone, diazepam, estradiol, estriol, hydroxyprogesterone, methimazole, phenobarbital, prednisone, progesterone, ritodrine, scopolamine, testosterone

Interfering: prednisolone

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: saliva

Sample preparation: 0.5 mL Saliva + 0.5 mL water + 1 mL mobile phase A, filter, inject a 400 μ L aliquot onto column A and elute to waste with mobile phase A, after 7 min elute the contents of column A onto column B with mobile phase A, after 8 min remove column A from the circuit and elute column B with mobile phase B, start the gradient, monitor the effluent from column B.

HPLC VARIABLES

Column: A 100 \times 4.6 Capcell pak MF [PCMF, silicone polymer-coated silica with diol and phenyl groups] (Shiseido); B Capcell pak CN (Shiseido)

Mobile phase: A MeCN:water 10:90 containing 2 mM trisodium citrate, adjusted to pH 6.5 with HCl; B Gradient. X was MeCN:water 10:90. Y was MeCN. X:Y from 100:0 to 72.3:27.7 over 5 min, maintain at 72.3:27.7 for 16 min, return to initial conditions over 1 min.

Column temperature: 40

Flow rate: A 0.5; B 0.5

Injection volume: 400

Detector: F ex 488 (10 mW Ar⁺ laser) em 537 following post-column reaction. The column effluent mixed with concentrated sulfuric acid pumped at 0.75 mL/min and flowed through a 2.5 m \times 0.25 mm i.d. Dyflon reaction coil at 105° to the detector.

CHROMATOGRAM

Retention time: 29.4

Limit of quantitation: 0.5 nM

KEY WORDS

column-switching; heart-cut; post-column reaction

REFERENCE

Okumura, T.; Nakajima, Y.; Takamatsu, T.; Matsuoka, M. Column-switching high-performance liquid chromatographic system with a laser-induced fluorimetric detector for direct, automated assay of salivary cortisol. *J.Chromatogr.B*, **1995**, *670*, 11–20

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 4.1 10 μm Versapak C18 (Alltech)

Mobile phase: MeCN:water 40:60

Flow rate: 1

Injection volume: 50

Detector: UV 254

KEY WORDS

for hydrocortisone and hydrocortisone acetate

REFERENCE

Michniak, B.B.; Player, M.R.; Sowell, J.W. Synthesis and *in vitro* transdermal penetration enhancing activity of lactam N-acetic acid esters. *J.Pharm.Sci.*, **1996**, *85*, 150–154

SAMPLE

Matrix: solutions

Sample preparation: Add 3 mL of a chloroform solution to 300 μL EtOH, add 1 mL 3 mg/mL acenaphthene-5-sulfonyl hydrazine in EtOH:toluene 10:90, evaporate to dryness under reduced pressure at 60°, reconstitute with 200 μL mobile phase, inject an aliquot. (Preparation of acenaphthene-5-sulfonyl hydrazine 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. Cool a solution of 1 g acenaphthene-5-sulfonyl chloride in 3 mL THF to 10° and pass nitrogen through the solution, add 400 μL 85% hydrazine hydrate dropwise with stirring (Caution! Hydrazine hydrate is a carcinogen!), maintain the temperature between 10° and 15°, stir for a further 15 min. Filter the upper THF layer through Celite, wash the Celite with 1 mL THF. Stir the filtrate vigorously and add two 10 mL portions of water, cool in a refrigerator for 1 h, filter the precipitate, wash with water, dry, recrystallize from EtOH to give acenaphthene-5-sulfonyl hydrazine (mp 132–4°).)

HPLC VARIABLES

Column: 500 × 1 10 μm silica

Mobile phase: Toluene:dioxane 90:10 (Caution! Dioxane is a carcinogen!)

Detector: F ex 230 em 350

OTHER SUBSTANCES

Simultaneous: fluocinolone acetonide

KEY WORDS

derivatization; normal phase

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: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil phenyl

Mobile phase: Gradient. Carbon dioxide:MeOH from 98:2 to 78:22 over 40 min.

Column temperature: 50

Flow rate: 2

Detector: UV

CHROMATOGRAM

Retention time: 12.1

OTHER SUBSTANCES

Simultaneous: estradiol, estriol, hydroxyprogesterone, norethisterone, testosterone, other steroids

KEY WORDS

SFC; 200 bar

REFERENCE

Hanson, M. Aspects of retention behaviour of steroids in packed column supercritical fluid chromatography. *Chromatographia*, **1995**, *40*, 58–68

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: 34

OTHER SUBSTANCES

Simultaneous: cortisone, dehydrocorticosterone, methylprednisolone, prednisone, tetrahydrocortisol, tetrahydrocortisone

Interfering: prednisolone

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 μ L aliquot of a 100 ppm solution.

HPLC VARIABLES**Column:** 150 \times 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 $^{\circ}$, ion source chamber 400 $^{\circ}$, discharge electrode, skimmer 1 aperture 300 μ m, skimmer 2 aperture 400 μ m, no nebulizer gas

CHROMATOGRAM**Retention time:** 6

OTHER SUBSTANCES**Simultaneous:** corticosterone, cortisone, progesterone

REFERENCENojima, 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

HPLC VARIABLES**Column:** 10 μ m Alltech octadecylsilyl**Mobile phase:** MeCN:water 45:55**Flow rate:** 1.5**Detector:** UV 242

KEY WORDS

water

REFERENCEPhares, K.; Cho, M.; Johnson, K.; Swarbrick, J. Drug transport across nylon 610 films: Influence of synthesis variables. *Pharm.Res.*, **1995**, 12, 248-256

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 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, aprobar-

bital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazeponide, 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, dapson, 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, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, 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, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelenamine, 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' 1.094

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (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: Condition a Bond Elut C18 SPE cartridge with 4 mL water then 3 mL MeOH. Add aqueous steroid solution to the SPE cartridge, elute with MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 μ m Nucleosil C18

Mobile phase: MeOH:water 70:30

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Limit of detection: 3.5 μ g/mL

OTHER SUBSTANCES

Also analyzed: prednisolone

KEY WORDS

SPE; for hydrocortisone or hydrocortisone 21-acetate

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

Sample preparation: Evaporate solution (eluate from preparative HPLC) to dryness under a stream of nitrogen, reconstitute with 10 μ L 2 μ g/mL 9-anthroylnitrile (Wako) in MeCN and 10 μ L triethylamine:MeCN 30:70 under nitrogen, let stand at room temperature for 20 min, add 5 μ L water, after 6 min add 50 μ L 600 mM acetic acid in MeOH, evaporate to dryness under a stream of nitrogen at 37°, reconstitute with 90 μ L MeOH: 0.4 N NaH₂PO₄ 60:40, add to a Cyclobond 1 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 onto column A and elute to waste with mobile phase, after the solvent front has passed through divert the effluent from column A onto 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: 5.48

Limit of detection: 9 pg

OTHER SUBSTANCES

Simultaneous: cortisone, 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: Sample + 400 μ L 5 mM DBD-PZ + 70 mM diethylphosphorocyanide in MeCN, react for 6 h, inject a 1 μ L 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 DBD-PZ as orange crystals, mp 121-2°.)

HPLC VARIABLES

Column: 150 × 4.6 5 μ m 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: 13 (hydrocortisone succinate)

Limit of detection: 14 fmol

OTHER SUBSTANCES

Simultaneous: alprostadil, dinoprost, prednisolone succinate

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:** 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 240

CHROMATOGRAM**Retention time:** 8 (hydrocortisone acetate)

OTHER SUBSTANCES**Simultaneous:** methyltestosterone, norethindrone, prednisolone, prednisolone succinate, prednisone, progesterone

REFERENCERoos, 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 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' 1.27

REFERENCERoos, 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 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: 16 (hydrocortisone), 10 (hydrocortisone acetate)

OTHER SUBSTANCES

Simultaneous: 4-androstene-3,11,17-trione, cortisone, cortisone acetate

KEY WORDS

normal phase

REFERENCE

Kane, M.P.; Tsuji, K. Radiolytic degradation scheme for ⁶⁰Co-irradiated corticosteroids. *J.Pharm.Sci.*, **1983**, *72*, 30–35

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 500 × 1 C18 (Alltech)

Mobile phase: MeOH:water 65:35

Flow rate: 0.04

Injection volume: 0.5

Detector: UV 254; MS, Hewlett Packard 5985, home-made interface (details in paper)

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Simultaneous: betamethasone

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: tissue

Sample preparation: Extract 70-125 mg tissue four times with 5 mL portions of ether: chloroform 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 80 mm long 10 μm octadecylsilane radial compression (Radial-Pak) (Waters)

Mobile phase: Gradient. A was MeOH:water 50:50. B was MeOH. A:B from 100:0 to 70:30 over 20 min, to 0:100 over 20 min.

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Extracted: androstenedione, deoxycortisol, 17-hydroxyprogesterone, testosterone

Simultaneous: estradiol, estriol, pregnenolone, progesterone, testosterone enanthate, testosterone propionate

KEY WORDS

tumor

REFERENCE

Kessler, M.J. Analysis of steroids from normal and tumor tissue by HPLC. *Clin.Chim.Acta*, **1982**, *125*, 21-30

SAMPLE

Matrix: urine

Sample preparation: 10 mL Urine + 40 μ L 25 μ g/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 μ L MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m 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: 13.6

Internal standard: corticosterone (17.8)

OTHER SUBSTANCES

Extracted: cortisone

REFERENCE

Lee, Y.S.; Lorenzo, B.J.; Koufis, T.; Reidenberg, M.M. Grapefruit juice and its flavonoids inhibit 11 β -hydroxysteroid dehydrogenase. *Clin.Pharmacol.Ther.*, **1996**, *59*, 62-71

SAMPLE

Matrix: urine

Sample preparation: Inject 500 μ L urine 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 and start the gradient, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 μ m Ultrabiosep C18 SFCC (ISRP); B 250 \times 4.6 5 μ m Ultrabase C18 SFCC

Mobile phase: A water; B Gradient. MeCN:water 20:80 for 10 min, to 40:60 over 10 min, re-equilibrate with 20:80 for 5 min. (Re-equilibrate column A with mobile phase A for 5 min before the next injection.)

Flow rate: 1

Injection volume: 500

Detector: UV 242

CHROMATOGRAM

Retention time: 22.77

Limit of detection: 3 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: 6 β -hydroxycortisol

Simultaneous: corticosterone, cortisone, deoxycorticosterone, 11-deoxycortisol, prednisolone, prednisone

KEY WORDS

column-switching

REFERENCE

Bidart, M.; Lesgards, G. Direct injection analysis of 6 β -hydroxycortisol and cortisol in urine by HPLC-UV with on-line IRSP column. *J.Liq.Chromatogr.*, **1995**, *18*, 725-738

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 100 ng methylprednisolone + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na₂HPO₄, 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 μ L MeOH, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Hypersil 5-ODS

Mobile phase: MeCN:water 30:70

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 245

CHROMATOGRAM

Retention time: 7

Internal standard: methylprednisolone (14)

Limit of detection: 51 pg

OTHER SUBSTANCES

Extracted: cortisone

Noninterfering: corticosterone, deflazacort, deoxycorticosterone, fluorocortisone acetate, 21-hydroxydeflazacort, 11 α -hydroxyprogesterone, prednisolone, prednisone, triamcinolone acetonide

Interfering: fluorocortisone

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: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na₂HPO₄, 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 μ L 5 μ g/mL IS in MeOH, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 6

Internal standard: methylprednisolone (9)

OTHER SUBSTANCES

Simultaneous: betamethasone, corticosterone, cortisone, dexamethasone, fluorocortisone acetate, hydroxyprogesterone, triamcinolone, triamcinolone acetonide

Interfering: fluorocortisone, prednisolone, prednisone

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: Equilibrate a Sephadex G-25M column with 100 mM pH 7.0 phosphate buffer. Condition a Bond-Elut C18 SPE cartridge with 1 mL MeCN, 4 mL acetone:water 20:80, and 4 mL water. 2 mL Urine + 500 μ L 500 mM pH 5.0 acetate buffer + 50 μ L 1 μ g/mL fludrocortisone in MeOH + 160 μ L 100000 Fishmann U/mL β -glucuronidase and 800000 Roy U/mL arylsulfatase (from *Helix pomatia*, Boehringer Mannheim), heat at 37° for 24 h, filter (0.45 μ m), add to the Sephadex column, wash with three 2 mL portions of 100 mM pH 7.0 phosphate buffer, elute with four 2 mL portions of 100 mM pH 7.0 phosphate buffer. Add the eluate to the SPE cartridge, wash with 4 mL water, wash with 4 mL acetone:water 20:80, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L MeOH, add 20 μ L cupric acetate solution, let stand at room temperature for 1 h, add 100 μ L reagent, heat at 60° for 40 min, cool, centrifuge briefly at 1000 g, inject a 100 μ L aliquot of the supernatant. (Cupric acetate solution was 0.7 g cupric acetate in 10 mL water diluted to 100 mL with MeOH. Reagent was 7 mM 1,2-diamino-4,5-methylenedioxybenzene in water containing 200 mM β -mercaptoethanol and 250 mM sodium hydrosulfite, store in the dark at 4°, stable for at least 2 weeks. Prepare 1,2-diamino-4,5-methylenedioxybenzene as follows. Add 5 g 1,2-(methylenedioxy)-4-nitrobenzene to 37.5 mL concentrated nitric acid and 12.5 mL glacial acetic acid, pour the yellow-colored solution into water, recrystallize the 1,2-dinitro-4,5-methylenedioxybenzene from EtOH (Rec.Trav.Chim.Pays-Bas 1930, 49, 45). Dissolve 5 g 1,2-dinitro-4,5-methylenedioxybenzene in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 80 mesh iron powder, add 20 mL concentrated HCl in small portions over 1 h while heating the mixture under reflux. Reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.6 M NaOH, extract three times with 200 mL portions of benzene. Combine the extracts, evaporate to dryness to give 1,2-diamino-4,5-methylenedioxybenzene, mix with 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-methylenedioxybenzene dihydrochloride, mp 176-9° (Chem.Pharm.Bull. 1987, 35, 687).)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m L-Column ODS (Chemicals Inspection and Testing Institute, Tokyo)

Mobile phase: MeOH:MeCN:500 mM ammonium acetate 50:10:40 (After each injection wash with MeOH:water 80:20 for 20 min, re-equilibrate for 20 min.)

Flow rate: 1

Injection volume: 100

Detector: F ex 350 em 390

CHROMATOGRAM

Retention time: 38.5

Internal standard: fludrocortisone (35.6)

Limit of detection: 1.18 ng/mL

OTHER SUBSTANCES

Extracted: aldosterone, tetrahydroaldosterone

Noninterfering: corticosterone, cortisone, hydroxycorticosteroids

KEY WORDS

SPE; derivatization

REFERENCE

Yoshitake, T.; Ishida, J.; Sonezaki, S.; Yamaguchi, M. High performance liquid chromatographic determination of 3 α ,5 β -tetrahydroaldosterone and cortisol in human urine with fluorescence detection. *Biomed.Chromatogr.*, **1992**, *6*, 217–221

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 1.5 μ g betamethasone + 100 mg K₂HPO₄ + 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 μ L MeOH, filter (0.45 μ m), inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m 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: 11.32

Internal standard: betamethasone (12.83)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 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: Adjust pH of 2 mL urine to 6.5, add 100 μ L 500 Fishman U/mL β -glucuronidase (from *E. coli*), add 200 μ L 200 mM pH 6.5 phosphate buffer, add 1 drop

chloroform, mix well, heat at 37° for 24 h, add 20 µL 100 µg/mL betamethasone in MeOH, add 4 mL dichloromethane, shake for 3 min. Remove the organic layer and wash it with 500 µL 100 mM NaOH, wash with 500 µL water. Remove the organic layer and evaporate it to dryness at 80°, reconstitute the residue in 100 µL mobile phase, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 µm Finepak C18

Mobile phase: MeOH:water 50:50

Column temperature: 40

Flow rate: 0.8

Injection volume: 10

Detector: F ex 370 em 480 following post-column reaction. The column effluent mixed with 400 mM NaOH and reagent pumped at 0.5 mL/min and the mixture flowed through a 30 m × 0.5 mm ID PTFE coil at 95° and another coil immersed in water to the detector. (Reagent was 0.5% benzamidine hydrochloride in isopropanol:water 50:50.)

CHROMATOGRAM

Retention time: 18

Internal standard: betamethasone (20)

OTHER SUBSTANCES

Simultaneous: tetrahydrocortisol, tetrahydrocortisone, tetrahydro-11-deoxycortisol

Noninterfering: aldosterone, androsterone, corticosterone, dehydroepiandrosterone, 11-deoxycorticosterone, 16-hydroxydehydroepiandrosterone, progesterone

KEY WORDS

post-column reaction

REFERENCE

Seki, T.; Yamaguchi, Y. New fluorimetric determination of 17-hydroxycorticosteroids after high-performance liquid chromatography using post-column derivatization with benzamidine. *J.Chromatogr.*, **1984**, *305*, 188–193

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Dolezalova, M. Routine high-performance liquid chromatographic determination of urinary unconjugated cortisol using solid-phase extraction and ultraviolet detection. *Clin.Chim.Acta*, **1994**, *231*, 129–137

Inoue, S.; Inokuma, M.; Harada, T.; Shibutani, Y.; Yoshitake, T.; Charles, B.; Ishida, J.; Yamaguchi, M. Simultaneous high-performance liquid chromatographic determination of 6β-hydroxycortisol and cortisol in urine with fluorescence detection and its application for estimating hepatic drug-metabolizing enzyme induction. *J.Chromatogr.B*, **1994**, *661*, 15–23 [extracted 6β-hydroxycortisol; urine; fluorescence detection; fludrocortisone (IS); gradient; LOD 0.95 ng/mL; human; monkey]

Lykkesfeldt, J.; Loft, S.; Poulsen, H.E. Simultaneous determination of urinary free cortisol and 6β-hydroxycortisol by high-performance liquid chromatography to measure human CYP3A activity. *J.Chromatogr.B*, **1994**, *660*, 23–29 [urine; extracted 6β-hydroxycortisol; dexamethasone (IS); SPE; gradient; LOQ 1 ng/mL]

Valvo, L.; Paris, A.; Savella, A.L.; Gallinella, B.; Ciranni Signoretti, E. General high-performance liquid chromatographic procedures for the rapid screening of natural and synthetic corticosteroids. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 805–810 [gradient; reverse phase; normal phase; for hydrocortisone, hydrocortisone 21-acetate, hydrocortisone 21-hemisuccinate; also beclomethasone, beclometha-

- sone 17,21-dipropionate, betamethasone, betamethasone 21-acetate, betamethasone 17,21-dipropionate, betamethasone 21-disodium phosphate, betamethasone 17-valerate, cortisone, cortisone 21-acetate, 11-deoxycorticosterone 21-acetate, dexamethasone, dexamethasone 21-acetate, dexamethasone 21-disodium phosphate, fluocinolone, fluocinolone acetonide, 9 α -fluorohydrocortisone 21-acetate, 9 α -fluorohydrocortisone, 9 α -fluoroprednisolone, 9 α -fluoroprednisolone 21-acetate, 6 α -methylprednisolone, 6 α -methylprednisolone 21-acetate, 6 α -methylprednisolone 21-sodium succinate, prednisolone, prednisolone 21-acetate, prednisolone 21-disodium phosphate, prednisolone 21-pivalate, prednisolone 21-sodium succinate, prednisone, triamcinolone, triamcinolone acetonide]
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- Shalaby, A.; Shahjahan, M. Improved high performance liquid chromatographic method for the determination of some corticosteroids. *J.Liq.Chromatogr.*, **1991**, *14*, 1267–1274 [formulations; ointment; lotion; tablets; injections; simultaneous dexamethasone, prednisolone]
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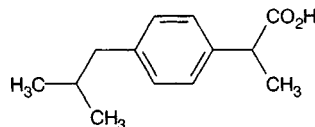
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Ibuprofen

Molecular formula: C₁₃H₁₈O₂

Molecular weight: 206.3

CAS Registry No.: 15687-27-1, 58560-75-1 (± mixture), 61054-06-6 (Al salt), 112017-99-9 (piconol)



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 50 μ L 2 μ g/mL flurbiprofen + 25 μ L 2 M HCl, vortex for 15 s, add 2 mL isooctane:isopropanol 85:15, rotate for 5 min, centrifuge at 3000 rpm for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 25 μ L 5 mg/mL 5-bromoacetyl acenaphthene in MeCN, add 10 μ L 3% triethylamine in MeCN, vortex for 30 s, heat at 75° for 5 min, evaporate to dryness under reduced pressure, reconstitute with 25 μ L MeCN, inject a 20 μ L aliquot. (Prepare 5-bromoacetyl acenaphthene as follows. Add 43 g bromoacetylchloride to 43 g acenaphthene dissolved in 200 mL dichloroethane, cool to -5° in an ice/salt bath, stir vigorously and add 38 g aluminum chloride in small portions over 90 min, do not allow temperature to go above 3°, place under reduced pressure for 30 min, add an excess of crushed ice. Separate the dichloroethane layer and wash it with two 100 mL portions of dilute HCl, wash with 100 mL 5% sodium carbonate solution. Dry the organic layer over anhydrous magnesium sulfate, remove the solvent under reduced pressure, allow the oily residue to solidify, remove liquid by blotting with filter paper. Purify the solid by chromatography on a 300 \times 20 column of 60-120 mesh silica gel, elute with toluene, unreacted acenaphthene elutes first followed by 5-bromoacetyl acenaphthene (mp 87-90°).)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Hypersil C18

Mobile phase: MeCN:water 90:10

Flow rate: 1

Injection volume: 20

Detector: F ex 250 em 450

CHROMATOGRAM

Internal standard: flurbiprofen

Limit of detection: 2.5 pmole

KEY WORDS

rat; plasma; protect from light; pharmacokinetics; 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: blood

Sample preparation: 500 μ L Plasma + 125 μ L 40 mM decanoic acid in MeCN, mix. Dialyze a 100 μ L sample against 20 mM pH 7.0 phosphate buffer using a Gilson Cuprophane membrane (molecular mass cut-off 15 kDa). Continuously pump the buffer through the dialysis cell and through column A at 3 mL/min for 9.6 min, backflush the contents of column A onto column B with the mobile phase, monitor the effluent from column B. (After each injection flush plasma channel with 1 mL 0.05% Triton X-100, with 1 mL 1 mM HCl, and with 2 mL water. After each injection flush buffer channel with 3 mL 20 mM pH 7.0 phosphate buffer and condition column A with 1 mL 20 mM pH 7.0 phosphate buffer.)

HPLC VARIABLES

Column: A $10 \times 240 \mu\text{m}$ Bondesil C18 (Analytichem); B $250 \times 3.15 \mu\text{m}$ C18 (RoSil Research Separation Laboratories)

Mobile phase: MeCN:MeOH:20 mM pH 3.2 phosphate buffer 50:10:40

Flow rate: 1

Injection volume: 100

Detector: UV 264

CHROMATOGRAM

Retention time: 11

Limit of detection: $2 \mu\text{g/mL}$

OTHER SUBSTANCES

Extracted: fenoprofen (UV 272), flurbiprofen (UV 247), ketoprofen (UV 261), naproxen (UV 272)

KEY WORDS

plasma; dialysis; column-switching

REFERENCE

Herráez-Hernández, R.; Van de Merbel, N.C.; Brinkman, U.A.T. Determination of the total concentration of highly protein-bound drugs in plasma by on-line dialysis and column liquid chromatography: application to non-steroidal anti-inflammatory drugs. *J.Chromatogr.B*, **1995**, 666, 127–137

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 1 M HCl + 100 μL fenoprofen solution + 10 mL ether, stir for 10 min, centrifuge at 6000 rpm for 5 min, repeat extraction 3 more times. Combine the organic layers and evaporate them to dryness under reduced pressure (0.5 bar), reconstitute the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: $4 \times 45 \mu\text{m}$ Licrospher 100 RP-18

Column: $125 \times 45 \mu\text{m}$ Licrospher 100 RP-18

Mobile phase: MeCN:pH 4.8 sodium acetate buffer 40:60

Injection volume: 50

Detector: UV 223

CHROMATOGRAM

Internal standard: fenoprofen

Limit of detection: LOD 5 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kleinbloesem, C.H.; Ouwerkerk, M.; Spitznagel, W.; Wilkinson, F.E.; Kaiser, R.R. Pharmacokinetics and bioavailability of percutaneous ibuprofen. *Arzneimittelforschung*, **1995**, 45, 1117–1121

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 100 μL 2.5 $\mu\text{g/mL}$ S-(+)-naproxen + 500 μL 600 mM sulfuric acid + 15 mL dichloromethane, mix for 20 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL 50 mM triethylamine in MeCN +

50 μ L 60 mM ethyl chloroformate in MeCN, vortex for 30 s, add 50 μ L 100 mM L-leucinamide in MeOH:triethylamine 100:14, let stand for 2 min, add 50 μ L water, inject a 10-50 μ L aliquot of the reaction mixture.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrabase C18 (Shandon)

Mobile phase: MeCN:60 mM KH_2PO_4 :triethylamine 49:51:0.1

Flow rate: 1.8

Injection volume: 10-50

Detector: UV 225

CHROMATOGRAM

Retention time: 5.5 (R(-)), 5.8 (S(+))

Internal standard: S-(+)-naproxen (2.5)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: flurbiprofen (UV 275), ketoprofen (UV 275)

KEY WORDS

plasma; chiral; derivatization

REFERENCE

Péhourcq, F.; Lagrange, F.; Labat, L.; Bannwarth, B. Simultaneous measurement of flurbiprofen, ibuprofen, and ketoprofen enantiomer concentrations in plasma using L-leucinamide as the chiral coupling component. *J.Liq.Chromatogr.*, **1995**, *18*, 3969-3979

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 20 μ L 500 μ g/mL phenylbutazone in MeOH + 1.5 mL MeOH, vortex, centrifuge for 15 min at 3000 g. Remove the supernatant and evaporate it to 500 μ L using a vortex evaporator, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: present but not specified

Column: 10 μ m RP-8 (Alltech)

Mobile phase: MeOH:1% acetic acid 78:22

Injection volume: 20

Detector: UV 235

CHROMATOGRAM

Internal standard: phenylbutazone

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: ibudice

KEY WORDS

dog; plasma; pharmacokinetics

REFERENCE

Samara, E.; Avnir, D.; Ladkani, D.; Bialer, M. Pharmacokinetic analysis of diethylcarbonate prodrugs of ibuprofen and naproxen. *Biopharm. Drug Dispos.*, **1995**, *16*, 201-210

SAMPLE

Matrix: blood

Sample preparation: Erythrocytes. 500 μL Erythrocytes + 5 μL 1 mg/mL indomethacin in MeOH + 900 μL water, shake for 5 min, sonicate for 5 min, let stand at room temperature for 5 min, add 400 μL 3 M HCl, shake for 5 min, add 6 mL dichloromethane, shake, centrifuge at 1930 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 100 μL mobile phase, inject a 10 μL aliquot. Plasma. 500 μL Plasma + 5 μL 1 mg/mL indomethacin in MeOH, acidify gradually with 900 μL 1 M HCl, shake, add 200 μL 3 M HCl, shake for 5 min, add 6 mL dichloromethane, shake, centrifuge at 1930 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 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 \times 3.3 5 μm C18 glass column (Tessek)

Mobile phase: MeOH:water 66:30 adjusted to pH 3.0 with 5% perchloric acid

Flow rate: 1.3

Injection volume: 10

Detector: UV 222

CHROMATOGRAM

Retention time: 9.9

Internal standard: indomethacin (7.8)

Limit of detection: 20 (plasma), 30 (erythrocytes) ng/mL

OTHER SUBSTANCES

Simultaneous: diazepam, phenylanthranilic acid

KEY WORDS

plasma; erythrocytes; rabbit; pharmacokinetics

REFERENCE

Sochor, J.; Klimes, J.; Sedláček, J.; Zahradnicek, M. Determination of ibuprofen in erythrocytes and plasma by high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 899–903

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 \times 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: 10.58

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lisinopril, loperamide, loprozepam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephensesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozone, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, propguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vandesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: alpidem, chlorambucil, floctafenine, ibuprofen, lidoflazine

KEY WORDS

whole blood; plasma

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 + 100 μL 200 $\mu\text{g}/\text{mL}$ tridecanoic acid in ethylene chloride + 200 μL 600 mM sulfuric acid + 3 mL isoctane:isopropanol 95:5, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 1 mL 2.4 mg/mL 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in ethylene chloride, add 5 mL reagent, reflux for 10 min, dilute with 10 mL ethylene chloride, wash with an equal volume of 200 mM NaOH, wash with an equal volume of 1 M HCl, wash with an equal volume of water. Remove the organic layer and dry it over sodium sulfate, evaporate to dryness, reconstitute the residue in mobile phase, inject an aliquot. (Prepare reagent by dissolving 5 mg p-nitrobenzylamine hydrochloride in 5 mL 200 mM NaOH, extract

with 5 mL ethylene chloride, dry the organic layer over anhydrous sodium sulfate, use this solution as the reagent.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm (R)-(-)-(1-naphthyl)ethylurea (Prepare by pumping 2 g (R)-(-)-1-(1-naphthyl)ethyl isocyanate in 100 mL dichloromethane through a 100 × 4.6 3 μm aminopropyl-silvanized silica column (Regis) at 2 mL/min (without detector), after 12.5 min recycle the mobile phase, after 2 h wash the column with 300 mL dichloromethane at 2 mL/min, wash with hexane:isopropanol 80:20 until a steady baseline is achieved.)

Mobile phase: Hexane:isopropanol 87.5:12.5

Flow rate: 1.5

Detector: UV 235

CHROMATOGRAM

Retention time: 21 (S), 22.5 (R)

Internal standard: tridecanoic acid (19)

Limit of quantitation: 2.5 μg/mL

KEY WORDS

derivatization; dog; chiral; plasma; pharmacokinetics

REFERENCE

Ahn, H.-Y.; Shiu, G.K.; Trafton, W.F.; Doyle, T.D. Resolution of the enantiomers of ibuprofen; comparison study of diastereomeric method and chiral stationary phase method. *J.Chromatogr.B*, **1994**, *653*, 163-169

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 50 ng flurbiprofen + 150 μL 1 M phosphoric acid + 5 mL hexane:ether 80:20, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 4 μm Nova Pak C18

Mobile phase: MeCN:water:acetic acid 59:40.5:0.5

Flow rate: 1.3

Detector: UV 233

CHROMATOGRAM

Internal standard: flurbiprofen

Limit of detection: 400 ng/mL

KEY WORDS

pharmacokinetics; plasma

REFERENCE

al-Meshal, M.A.; El-Sayed, Y.M.; al-Balla, S.R.; Gouda, M.W. The effect of colestipol and cholestyramine on ibuprofen bioavailability in man. *Biopharm.Drug Dispos.*, **1994**, *15*, 463-471

SAMPLE

Matrix: blood

Sample preparation: 10 μL Plasma is injected into MeCN pumped at 0.2 mL/min, precipitated proteins are removed by 0.5 and 0.2 μm filters in series, MeCN containing sample is switched into mobile phase allowed to pass onto analytical column. Next filter unit is switched out of circuit and back-flushed to waste with 100 mM sodium dodecyl sulfate at

2 mL/min, analytical column is eluted in normal fashion with mobile phase. Equilibrate filters with MeCN for 5 min before next injection.

HPLC VARIABLES

Guard column: 20 mm Brownlee C18

Column: 250 × 4.6 5 µm Ultrasphere C18

Mobile phase: MeCN:buffer 70:30 (Buffer was 0.094% triethylamine in water adjusted to pH 3 with glacial acetic acid.)

Flow rate: 1

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 6.39

KEY WORDS

plasma; dog

REFERENCE

Asafu-Adjaye, E.B.; Su, S.Y.; Shiu, G.K. Switching-valve-filter technique for the direct injection and analysis of drugs in plasma using high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *652*, 35–42

SAMPLE

Matrix: blood

Sample preparation: Inject sample 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 for 2 min, elute column B with mobile phase B, monitor effluent from column B.

HPLC VARIABLES

Column: A 10 × 3 BioTrap Acid C18 (ChromTech); B 10 × 3 CT-sil C18 guard column + 100 × 4.6 5 µm CT-sil C18 (ChromTech)

Mobile phase: A 200 mM pH 2.1 phosphate buffer; B MeOH:82 mM pH 6.0 phosphate buffer 65:35

Flow rate: A 0.55; B 1

Injection volume: 10

Detector: F ex 225 em 535

CHROMATOGRAM

Retention time: 5.8

Limit of quantitation: 520 ng/mL

KEY WORDS

plasma; column-switching; direct injection

REFERENCE

Hermansson, J.; Grahn, A. Determination of drugs by direct injection of plasma into a biocompatible extraction column based on a protein-entrapped hydrophobic phase. *J.Chromatogr.A*, **1994**, *660*, 119–129

SAMPLE

Matrix: blood

Sample preparation: Place 100 µL 100 µM diclofenac in dichloromethane in the bottom of a tube and evaporate it to dryness under a stream of nitrogen, add 100 µL plasma, add 25 µL 1 M HCl, mix, add to a dry Chem Elut diatomaceous earth SPE cartridge (Varian), let stand for 5 min, elute with 6 mL n-hexane:diethyl ether:isopropanol 50:

50:1. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L 2 mM (-)-APMB in dichloromethane, add 100 μ L 20 mM 2,2'-dipyridyl disulfide in dichloromethane, add 100 μ L 20 mM triphenylphosphine in dichloromethane, mix, let stand at room temperature for 5 min. Evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 400 μ L mobile phase, inject a 10 μ L aliquot. ((-)-APMB is (-)-2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole. Synthesis is as follows. Hydrogenate 5-methoxy-2-nitrophenol in EtOH over platinum oxide to give 2-amino-5-methoxyphenol (J. Org. Chem. 1957, 22, 220). It should be possible to prepare ethyl 4-acetylbenzimidate hydrochloride ($\text{CH}_3\text{COC}_6\text{H}_4\text{C}(=\text{NH})\text{OC}_2\text{H}_5\cdot\text{HCl}$) by passing dry hydrogen chloride into a mixture of 4-acetylbenzoxazole and 1.2-1.5 equivalents EtOH in an inert solvent (e.g., benzene, chloroform, dioxane, ether, nitrobenzene (Caution! Benzene, chloroform, and dioxane are carcinogens!) at 0-5°, the benzimidate should crystallize from the mixture in 7-10 days (J. Chem. Soc. 1942, 103). Add a solution of 5.5 g 2-amino-5-methoxyphenol in 200 mL MeOH to 9 g ethyl 4-acetylbenzimidate hydrochloride, stir at 60-70° for 4 h, evaporate to dryness under reduced pressure, recrystallize from EtOH to give 4-(6-methoxy-2-benzoxazolyl)acetophenone as fine orange-yellow crystals (mp 167°) (J. Chromatogr. 1990, 532, 65). Add 7.0 g hydroxylamine hydrochloride and 8.2 g sodium acetate to 10.1 g 4-(6-methoxy-2-benzoxazolyl)acetophenone in 500 mL EtOH:water 95:5, reflux for 1 h, pour into ice-water, filter, recrystallize from EtOH:water 90:10 to give 4-(6-methoxy-2-benzoxazolyl)acetophenone oxime as faint reddish needles (mp 212°). Dissolve 4.7 g 4-(6-methoxy-2-benzoxazolyl)acetophenone oxime in 300 mL MeOH, add 3 g 10% palladium on charcoal, add 10.5 g ammonium formate, reflux for 30 min, filter, evaporate the filtrate to dryness under reduced pressure. Take up the residue in 100 mL 5% HCl and wash the aqueous phase with 100 mL ethyl acetate. Adjust the pH of the aqueous layer to 13-14 with 10% NaOH and extract with 200 mL ethyl acetate. Wash the organic layer with 100 mL water and dry it over anhydrous sodium sulfate, evaporate to dryness under reduced pressure to give racemic 2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole. Dissolve 3.6 g racemic 2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole in 50 mL EtOH and add 3.5 g (S)-(-)- α -methoxy- α -trifluoromethylphenylacetic acid, allow to stand overnight at 5°. Collect the precipitate and fractionally crystallize it from EtOH 4 times. Take up the final product in 5% NaOH and extract it with ethyl acetate, wash the organic layer with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from EtOH to give (-)-2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole as pale yellow crystals (mp 74°) (J. Chromatogr. 1993, 645, 75).)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m TSK gel ODS-80TS (Tosoh)

Mobile phase: MeCN:water:acetic acid 70:30:0.1

Column temperature: 40

Flow rate: 1

Injection volume: 10

Detector: F ex 320 em 380

CHROMATOGRAM

Retention time: 11.0 (S), 12.2 (R)

Internal standard: diclofenac (14.0)

Limit of quantitation: 200 ng/mL (S); 400 ng/mL (R)

KEY WORDS

derivatization; rat; plasma; chiral; pharmacokinetics; SPE

REFERENCE

Kondo, J.; Suzuki, N.; Naganuma, H.; Imaoka, T.; Kawasaki, T.; Nakanishi, A.; Kawahara, Y. Enantiospecific determination of ibuprofen in rat plasma using chiral fluorescence derivatization reagent, (-)-2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole. *Biomed. Chromatogr.*, **1994**, 8, 170-174

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 the SPE cartridge, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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, benzoylcegonine, 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, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: desipramine, 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: Condition a 1 mL 60 μ m Separon SGX C18 SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL buffer. 250 μ L Blood + 5 μ L 500 μ g/mL indomethacin in MeOH + 500 μ L water, shake for 5 min, sonicate for 5 min, let stand at room temperature for 5 min, add 1 mL buffer, shake for 5 min, centrifuge at 1930 g for 10 min, add the supernatant to the SPE cartridge, wash with 5 mL buffer, wash with 10 mL

water, dry with vacuum for 5 min, elute with dichloromethane. Evaporate eluate to dryness under a stream of nitrogen, dissolve in 100 μL mobile phase, inject 10 μL aliquot. (Buffer was 66 mM KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)

HPLC VARIABLES

Column: 150 \times 3.3 5 μm Separon SGX C18 glass column
Mobile phase: MeOH water 220:100, adjusted to pH 3.0 with 5% perchloric acid
Flow rate: 1.3
Injection volume: 10
Detector: UV 222

CHROMATOGRAM

Retention time: 9.9
Internal standard: indomethacin (7.8)
Limit of detection: 100 ng/mL
Limit of quantitation: 300 ng/mL

KEY WORDS

SPE; rabbit; human

REFERENCE

Sochor, J.; Klimes, J.; Zahradníček, M.; Sedláček, J. High-performance liquid chromatographic assay for ibuprofen in whole blood using solid-phase extraction. *J.Chromatogr.B*, **1994**, 654, 282–286

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Bond-Elut SPE cartridge with 4 mL MeOH, then 4 mL water, then 4 mL 10 mM phosphoric acid. 500 μL Plasma + 25 μL 80 $\mu\text{g}/\text{mL}$ ibufenac in MeOH, vortex 30 s, stand at RT for 10 min, add 1 mL MeCN, vortex 30 s, centrifuge at 1200 g. Remove supernatant and add it to 8.5 mL 10 mM pH 2 phosphoric acid. Add this solution to the SPE cartridge, wash with 2 mL MeCN:10 mM phosphoric acid 20:80, centrifuge at 1800 g for 3 min to remove liquid completely, elute with 1 mL MeCN:10 mM phosphoric acid 1:1, centrifuge at 1800 g for 3 min to remove all of eluent. Evaporate eluent to dryness under a stream of nitrogen at 37°, reconstitute in 400 μL MeCN:MeOH:1% acetic acid (pH 3) 10:15:75, sonicate 3 min, vortex 1 min, inject 100 μL aliquot

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μm Brownlee RP-18
Column: 150 \times 4.6 5 μm Axxiom ODS
Mobile phase: MeOH:10 mM pH 2.2 trifluoroacetic acid 57:43
Flow rate: 1.2
Injection volume: 100
Detector: UV 225; UV 214

CHROMATOGRAM

Retention time: 24.8
Internal standard: ibufenac (16.1)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, glucuronides
Noninterfering: aspirin, acetaminophen, salicylic acid

KEY WORDS

plasma; SPE

REFERENCE

Castillo, M.; Smith, P.C. Direct determination of ibuprofen and ibuprofen acyl glucuronide in plasma by high-performance liquid chromatography using solid-phase extraction. *J.Chromatogr.*, **1993**, *614*, 109-116

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 50 μ L 200 μ g/mL fenoprofen in MeOH:water 1:4 + 200 μ L 1 M sulfuric acid + 3 mL isoctane:isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and evaporate it to dryness. Add 300 μ L 50 mM triethylamine in MeCN and 50 μ L 6 mM ethyl chloroformate in MeCN, wait 30 s, add 25 μ L 0.1% (S)-naphthylethylamine in MeCN:triethylamine 98:2, after 3 min add 25 μ L 2.5% ethanolamine in MeCN, inject 2-30 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m Partisil ODS 3 RAC

Mobile phase: MeCN:water:acetic acid:triethylamine 60:40:0.1:0.02, final pH 5.0 (After every third injection flush with MeCN for 6 min at 1.6 mL/min, equilibrate with mobile phase for 9 min.)

Flow rate: 1.2

Injection volume: 2-30

Detector: F ex 280 em 320

CHROMATOGRAM

Retention time: 10.5 (S-(+)), 11.8 (R-(-))

Internal standard: fenoprofen (7.5 (S), 8.8 (R))

Limit of detection: 10 ng/mL

KEY WORDS

plasma; chiral; also UV 232 (Clin. Chem. 1988, 34 ,493)

REFERENCE

Lemko, C.H.; Caillé, G.; Foster, R.T. Stereospecific high-performance liquid chromatographic assay of ibuprofen: improved sensitivity and sample processing efficiency. *J.Chromatogr.*, **1993**, *619*, 330-335

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 500 μ L MeCN, vortex 2 min, centrifuge at 2000 g for 4 min. Remove supernatant and saturate it with anhydrous ammonium sulfate. Centrifuge at 2000 g for 2 min, inject 50-100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 3 μ m Spherisorb ODS

Mobile phase: MeCN:pH 2.2 phosphoric acid 50:50

Flow rate: 1

Injection volume: 50-100

Detector: UV 220

CHROMATOGRAM

Retention time: 13

Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, aspirin, 5-azacytidine, baclofen, cimetidine, cyclophosphamide, cyclosporin A, famotidine, 5-fluorouracil, ranitidine, verapamil

KEY WORDS

plasma

REFERENCE

Rustum, A.M. Assay of ibuprofen in human plasma by rapid and sensitive reversed-phase high-performance liquid chromatography: application to a single dose pharmacokinetic study. *J.Chromatogr.Sci.*, **1991**, 29, 16-20

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Serum + 50 μ L 1 M HCl, add 4 mL hexane:isopropanol 85:15, vortex for 30 s, centrifuge at 2000 g for 5 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 300 μ L MeCN:20 mM pH 3.5 phosphate buffer 40:60 containing 5 mM tetrabutylammonium bromide, inject a 200 μ L aliquot onto column A with mobile phase A, collect the eluate containing ibuprofen in a sample loop and inject it onto column B with mobile phase B. Collect the eluate containing ibuprofen in a sample loop and inject it onto column C with mobile phase C, monitor the effluent from column C.

HPLC VARIABLES

Column: A 70 \times 4.6 5 μ m YMC ODS A type (Yakamura Chemical); B 70 \times 4.6 5 μ m YMC ODS A type (Yakamura Chemical); C 150 \times 4.6 5 μ m TSK gel ODS 80 TM (Tosoh)

Mobile phase: A MeCN:20 mM pH 3.5 phosphate buffer 40:60 containing 5 mM tetrabutylammonium bromide; B MeCN:20 mM pH 7 phosphate buffer 30:70 containing 5 mM tetrabutylammonium bromide; C MeCN:20 mM pH 7 phosphate buffer 30:70

Column temperature: 40

Flow rate: 1

Injection volume: 200

Detector: UV 221

CHROMATOGRAM

Retention time: 41

Limit of detection: 0.5 ng/mL

KEY WORDS

serum; column-switching; heart-cut

REFERENCE

Yamashita, K.; Motohashi, M.; Yashiki, T. Column-switching techniques for high-performance liquid chromatography of ibuprofen and mefenamic acid in human serum with short-wavelength ultraviolet detection. *J.Chromatogr.*, **1991**, 570, 329-338

SAMPLE**Matrix:** blood

Sample preparation: 500 μ L Plasma + 200 μ L 2 M HCl + 6 mL ice-cold hexane:diethyl ether 8:2, extract, centrifuge at 1500 g for 10 min. Remove 5 mL of organic layer and evaporate it to dryness under a stream of nitrogen. Dissolve in 250 μ L isopropanol:water 2:8, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.0 AGP (EnantioPac)

Mobile phase: 20 mM pH 6.7 phosphate buffer containing 0.5% isopropanol and 5 mM dimethyloctylamine

Column temperature: 15

Flow rate: 0.5

Injection volume: 40

Detector: UV 220

CHROMATOGRAM

Retention time: 14(R), 17(S)

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: fenoprofen

KEY WORDS

plasma

REFERENCE

Menzel-Soglowek, S.; Geisslinger, G.; Brune, K. Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1 -acid glycoprotein column. *J.Chromatogr.*, **1990**, *532*, 295–303

SAMPLE

Matrix: blood

Sample preparation: Dialyze (Spectrum Medical Industries, Inc. Spectrophor 2, 12000-14000 molecular weight cutoff) 3.5 mL plasma with 3.5 mL buffer at 37° with one 6 cm oscillation per s for 16-17 h. Remove a 2 mL aliquot of the buffer and add it to 500 μ L 2 M sulfuric acid and 10 mL heptane:isopropanol 95:5, vortex for 1 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L 1% thionyl chloride in dichloromethane (freshly prepared), vortex briefly, heat at 70° in a tube securely sealed with a PTFE-lined cap for 1 h, let cool for 15 min, add 500 μ L 1% S(-)-1-phenylethylamine in dichloromethane (freshly prepared), vortex briefly, let stand at room temperature for 20 min, add 500 μ L 2 M sulfuric acid, add 5 mL heptane, vortex for 1 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, vortex briefly, inject a 50-90 μ L aliquot. (Buffer was isotonic pH 7.4 phosphate buffer prepared from 67 mM NaH₂PO₄, 67 mM Na₂HPO₄, and NaCl.)

HPLC VARIABLES

Column: 250 \times 4.5 μ m Hibar Lichrosorb Si60

Mobile phase: Heptane:isopropanol 97.5:2.5

Flow rate: 2

Injection volume: 50-90

Detector: UV 216

CHROMATOGRAM

Retention time: 3 (R-(-)), 7 (S-(+))

KEY WORDS

plasma; normal phase; derivatization; chiral

REFERENCE

Evans, A.M.; Nation, R.L.; Sansom, L.N.; Bochner, F.; Somogyi, A.A. Stereoselective plasma protein binding of ibuprofen enantiomers. *Eur.J.Clin.Pharmacol.*, **1989**, *36*, 283–290

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 100 μ L 100 μ g/mL IS in 10 mM NaOH + 200 μ L 600 mM sulfuric acid + 3 mL isoctane:isopropanol 95:5, vortex for 30 s, centrifuge at

1800 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L 50 mM triethylamine in MeCN, add 50 μ L 6 mM ethyl chloroformate in MeCN, after 30 s add 25 μ L 1 mL/L (S)-(-)-1-(1-naphthyl)ethylamine in MeCN, let stand for 3 min, add 500 μ L 250 mM HCl, add 2 mL chloroform, vortex for 15 s, centrifuge at 1800 g for 2 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μ L mobile phase, inject a 10-50 μ L aliquot.

HPLC VARIABLES

Guard column: 20 mm long 37-53 μ m reversed-phase

Column: 100 \times 4.6 5 μ m Partisil 5 ODS-3

Mobile phase: MeCN:water:acetic acid:triethylamine 55:45:0.1:0.02, pH 4.9

Flow rate: 1

Injection volume: 10-50

Detector: UV 232

CHROMATOGRAM

Retention time: 18.5 (S), 21.0 (R)

Internal standard: (\pm)-2-(4-benzoylphenyl)butyric acid (11, 13 (enantiomers))

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: etodolac (enantiomers not resolved), flurbiprofen, ketoprofen, tiaprofenic acid (not derivatized)

KEY WORDS

derivatization; plasma; chiral; pharmacokinetics

REFERENCE

Mehvar, R.; Jamali, F.; Pasutto, F.M. Liquid-chromatographic assay of ibuprofen enantiomers in plasma. *Clin.Chem.*, 1988, 34, 493-496

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply it to the SPE cartridge. Wash with 100 μ L water, elute with three 500 μ L portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 5 μ m Spherisorb ODS

Mobile phase: MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 250

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Simultaneous: acetaminophen, fenoprofen, indomethacin, ketoprofen, naproxen, salicylic acid

KEY WORDS

whole blood; SPE

REFERENCE

Moore, C.M.; Tebbett, I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis. *Forensic Sci.Int.*, **1987**, *34*, 155–158

SAMPLE

Matrix: blood

Sample preparation: 25 μ L Plasma + 50 μ L 40 μ g/mL mefenamic acid in MeCN, vortex 10 s, centrifuge at 11000 g for 2 min, inject a 20 μ L aliquot

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μ m Brownlee RP-18

Column: 250 \times 4.5 5 μ m IBM octadecyl

Mobile phase: MeCN:MeOH:water:85% phosphoric acid 58:5:37:0.05

Flow rate: 1.8

Injection volume: 20

Detector: UV 196

CHROMATOGRAM

Retention time: 6.8

Internal standard: mefenamic acid (9.8)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, caffeine, carbamazepine, chloramphenicol, desipramine, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid

KEY WORDS

plasma; rat

REFERENCE

Shah, A.; Jung, D. Improved high-performance liquid chromatographic assay of ibuprofen in plasma. *J.Chromatogr.*, **1985**, *344*, 408–411

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 100 μ g/mL tolmetin + 0.5 mL 1 M HCl + 10 mL dichloromethane, shake 10 min, centrifuge at 1000 g for 5 min. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute with 200 μ L mobile phase, inject 10-30 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 Partisil 10 ODS-3

Mobile phase: MeOH:water:phosphoric acid 700:300:1

Flow rate: 2.5

Injection volume: 10-30

Detector: UV 220

CHROMATOGRAM

Retention time: 7.7

Internal standard: tolmetin (3.2)

Limit of quantitation: 5 μ g/mL

KEY WORDS

plasma

REFERENCE

Lockwood, G.F.; Wagner, J.G. High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids. *J.Chromatogr.*, **1982**, *232*, 335–343

SAMPLE

Matrix: blood, synovial fluid

Sample preparation: 0.5 mL Plasma or synovial fluid + 50 μ L 300 μ g/mL flurbiprofen + 200 μ L 2 M HCl, vortex 15 s, add 5 mL hexane:diethyl ether 1:1, tumble 10 min on a rotary mixer, centrifuge at 10000 g for 5 min. Remove organic layer and evaporate it to dryness under vacuum centrifugation. Reconstitute residue in 300 μ L MeOH + 200 μ L water, sonicate 5 min, vortex 15 s, inject aliquot.

HPLC VARIABLES

Guard column: 20 \times 2 Perisorb RP18 30-40 μ m pellicular

Column: 125 \times 4.6 5 μ m Spherisorb ODS 1

Mobile phase: MeOH:water 65:35, adjusted to pH 3.3 with phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 8

Internal standard: flurbiprofen (6.5)

Limit of detection: <5 μ g/mL

KEY WORDS

plasma

REFERENCE

Blagbrough, I.S.; Daykin, M.M.; Doherty, M.; Patrick, 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. 1 mL Plasma + 100 μ L 1 M HCl + 100 μ L 250 μ g/mL flurbiprofen + 100 μ L water + 6 mL ether:hexane 20:80, shake for 10 min, centrifuge at 900 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 100 μ L 10 mM NaOH, sonicate for 3 min, add 50 μ L 100 mM pH 7.0 phosphate buffer containing 0.1% dimethyloctylamine, sonicate for 3 min, inject a 5 μ L aliquot. Urine. 1 mL Urine + 500 μ L 1 M NaOH, let stand for 30 min, add 700 μ L 1 M HCl, add 100 μ L 250 μ g/mL flurbiprofen, add 1 mL water, add 5 mL ether:hexane 20:80, shake for 10 min, centrifuge at 900 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 100 μ L 10 mM NaOH, sonicate for 3 min, add 50 μ L 100 mM pH 7.0 phosphate buffer containing 0.1% dimethyloctylamine, sonicate for 3 min, inject a 5 μ L aliquot. (To measure unconjugated ibuprofen in urine proceed as for plasma.)

HPLC VARIABLES

Guard column: 10 \times 3 5 μ m Chiral-AGP (ChromTech)

Column: 100 \times 4 5 μ m Chiral-AGP (ChromTech)

Mobile phase: Gradient. A was 10 mM pH 7.0 phosphate buffer containing 1 mM dimethyloctylamine. B was isopropanol:10 mM pH 7.0 phosphate buffer 50:50 containing 1 mM dimethyloctylamine. A:B 99.2:0.8 for 5 min, to 59:41 over 10 min, re-equilibrate for 10 min.

Flow rate: 0.9

Injection volume: 5

Detector: UV 220 for 7 min, then UV 245

CHROMATOGRAM

Retention time: 3.1 (R), 5.3 (S)

Internal standard: flurbiprofen (9.5, 11.6)

Limit of detection: 100 ng/mL

Limit of quantitation: 250 ng/mL

KEY WORDS

plasma; chiral

REFERENCE

de Vries, J.X.; Schmitz-Kummer, E.; Siemon, D. The analysis of ibuprofen enantiomers in human plasma by high-performance liquid chromatography on an α 1-acid glycoprotein chiral stationary phase. *J.Liq.Chromatogr.*, **1994**, *17*, 2127–2145

SAMPLE

Matrix: blood, urine

Sample preparation: 100 μ L Urine or rat plasma or 500 μ L human plasma + 50 μ L 25 μ g/mL fenoprofen in MeOH:10 mM NaOH 10:90 + 200 μ L 600 mM sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μ L 50 mM triethylamine in MeCN, add 50 μ L 6 mM ethyl chloroformate in MeCN, vortex for 30 s, add 50 μ L 500 mM R-(+)- α -phenylethylamine in MeCN:triethylamine 80:20, vortex briefly, let stand for 2 min, add 1 mL 250 mM HCl, add 3 mL chloroform, vortex for 30 s, centrifuge at 1800 g for 2 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μ L mobile phase, inject a 10-150 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 37-53 μ m reversed-phase

Column: 100 \times 4.6 5 μ m C18 (Phenomenex)

Mobile phase: MeCN:water:acetic acid:triethylamine 46.5:53.5:0.1:0.03, pH 4.9

Flow rate: 1.6

Injection volume: 10-150

Detector: UV 225

CHROMATOGRAM

Retention time: 15.69 (R), 17.65 (S)

Internal standard: fenoprofen (11.70, 13.40 (enantiomers))

Limit of quantitation: 250 ng/mL

KEY WORDS

derivatization; human; rat; plasma; chiral; pharmacokinetics

REFERENCE

Wright, M.R.; Sattari, S.; Brocks, D.R.; Jamali, F. Improved high-performance liquid chromatographic assay method for the enantiomers of ibuprofen. *J.Chromatogr.*, **1992**, *583*, 259–265

SAMPLE

Matrix: bulk

Sample preparation: 10 mg Compound + 10 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride + 2 drops 3,5-dimethylaniline + 1.5 mL dichloromethane, mix, after 30 min add 1 mL 1 M HCl, shake vigorously. Remove the lower organic layer and dry it over anhydrous magnesium sulfate, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm D N-(3,5-dinitrobenzoyl)phenylglycine (Regis)**Mobile phase:** Hexane:isopropanol 80:20**Flow rate:** 2**Injection volume:** 20**Detector:** UV 254; UV 280

CHROMATOGRAM**Retention time:** k' 1.23 (for first enantiomer)

OTHER SUBSTANCES**Also analyzed:** carprofen, cicloprofen, etodolac, fenoprofen, flurbiprofen, ketoprofen, naproxen, piroprofen, tiaprofenic acid

KEY WORDSderivatization; $\alpha = 1.30$; chiral

REFERENCEPirkle, W.H.; Murray, P.G. The separation of the enantiomers of a variety of non-steroidal anti-inflammatory drugs (NSAIDs) as their anilide derivatives using a chiral stationary phase. *J.Liq.Chromatogr.*, **1990**, *13*, 2123–2134

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:** 8.12 (A), 10.50 (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, 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, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, mida-

zolam, 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, pimizide, 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, sulfapyrazone, 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

some 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 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica

Mobile phase: Heptane:isopropanol:trifluoroacetic acid 98:2:0.1

Flow rate: 1

Injection volume: 1000

Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.19

KEY WORDS

chiral; $\alpha = 1.09$

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: solutions

HPLC VARIABLES

Column: 150 mm long 5 μ m Microsorb-MV C18

Mobile phase: MeCN:10 mM pH 7 phosphate buffer 34:66

Flow rate: 1.5

Detector: UV 220

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:** solutions**Sample preparation:** 1 mL 5 mM Ibuprofen in dichloromethane + 300 μ L 1 mg/mL hydroxybenzotriazole in dichloromethane:pyridine 99:1 + 300 μ L 11 mg/mL 1-ethyl-3-dimethylaminopropylcarbodiimide in dichloromethane + 300 μ L 3.47 mg/mL 1-naphthylamine (Caution! 1-Naphthylamine is a carcinogen!) in dichloromethane, vortex, let stand for 1 h, evaporate to dryness under a stream of nitrogen, reconstitute with 5 mL MeOH, inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 2.1 Tollycellulose EXP B101 (tris(4-methylbenzoate)cellulose covalently bonded to 10 μ m aminopropylsilica)**Mobile phase:** MeOH:buffer 85:15 (Buffer was 14.05 g/L sodium perchlorate adjusted to pH 2.0.)**Flow rate:** 0.21**Injection volume:** 1**Detector:** UV 230; UV 254

CHROMATOGRAM**Retention time:** k' 1.43 (first enantiomer)

OTHER SUBSTANCES**Also analyzed:** fenopufen, flurbiprofen, ketoprofen, tiaprofenic acid

KEY WORDSderivatization; narrow-bore; chiral; α = 1.31; see also *Biomed. Chromatogr.* 1995, 9, 292

REFERENCEVan Overbeke, A.; Baeyens, W.; Van Der Weken, G.; Van de Voorde, I.; Dewaele, C. Comparative chromatographic study on the chiral separation of the 1-naphthylamine derivative of ketoprofen on cellulose-based columns of different sizes. *Biomed. Chromatogr.*, 1995, 9, 289–290

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 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, dapson, debrisoquine, desipramine, dexamethasone, dex-

tromethorphan, 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, iminostilbene, imipramine, indomethacin, isocarboxtyril, 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, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methylidopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyriline, pyrilamine, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 Chiralpak AD (Daicel)

Mobile phase: Carbon dioxide:MeOH 96:4

Column temperature: 30

Flow rate: 2.5

Detector: UV 210

CHROMATOGRAM

Retention time: 4.3, 5 (enantiomers)

OTHER SUBSTANCES

Simultaneous: fenoprofen, flurbiprofen, ketoprofen, naproxen

KEY WORDS

SFC; 250 bar; chiral

REFERENCE

Kot, A.; Sandra, P.; Venema, A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs. *J.Chromatogr.Sci.*, **1994**, *32*, 439–448

SAMPLE**Matrix:** solutions

Sample preparation: Mix 1 mL 100 µg/mL compound in dichloromethane with 300 µL 100 µg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, 300 µL 1.1 mg/mL 1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride in dichloromethane, and 300 µL 300 µg/mL benzylamine in dichloromethane, vortex, let stand at room temperature for 1.5 h, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 500 µL MeOH, inject an aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 10 µm EXP B101 tris(4-methylbenzoate) cellulose on silica (Bio-Rad)**Mobile phase:** MeOH:buffer 70:30 (Prepare buffer solution by dissolving 14.05 g sodium perchlorate in water, adjust pH to 2.0, make up to 1 L with water.)**Flow rate:** 1**Detector:** UV 230**CHROMATOGRAM****Retention time:** 7 (R), 9 (S)**OTHER SUBSTANCES**

Also analyzed: benoxaprofen (MeOH:buffer 80:20), carprofen, fenoprofen, flurbiprofen, ketoprofen, piroprofen, tiaprofenic acid

KEY WORDS

derivatization; chiral

REFERENCE

Van Overbeke, A.; Baeyens, W.; Van den Bossche, W.; Dewaele, C. Separation of 2-arylpropionic acids on a cellulose based chiral stationary phase by RP-HPLC. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 901–909

SAMPLE**Matrix:** solutions

Sample preparation: Condition an Analytichem AASP propylbenzenesulfonic acid (SCX) SPE cartridge with isopropanol at 4 mL/min for 1.5 min and with mobile phase at 4 mL/min for 2 min. 100 µL Ibuprofen solution in dichloromethane + 100 µL 500 µg/mL 2-phenylpropionic acid in dichloromethane + 200 µL 100 mM triethylamine in dichloromethane + 100 µL 60 mM ethyl chloroformate, vortex for 15 s, let stand for 15 min, add 100 µL 500 mM p-anisidine in dichloromethane, vortex for 15 s, let stand for 5 min, add 600 µL isopropanol:hexane 10:90, vortex for 15 s, add a 25 µL aliquot to the SPE cartridge, elute the contents of the SPE cartridge onto the analytical column with mobile phase, after 2.43 min remove the SPE cartridge from the circuit, elute the analytical column with mobile phase and monitor the effluent from the column.

HPLC VARIABLES**Column:** 250 × 4.6 5 µm Rexchrom Regis Pirkle D-phenylglycine (Regis)**Mobile phase:** Hexane:isopropanol 90:10

Flow rate: 2
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 10 (S), 12.5 (R)
Internal standard: 2-phenylpropionic acid (16 (S), 18 (R))
Limit of detection: 500 ng/mL

KEY WORDS

chiral; derivatization; SPE

REFERENCE

Nicoll-Griffith, D.; Scartozzi, M.; Chiem, N. Automated derivatization and high-performance liquid chromatographic analysis of ibuprofen enantiomers. *J.Chromatogr.A*, **1993**, *653*, 253–259

SAMPLE

Matrix: solutions

Sample preparation: Dissolve the compound in 400 μ L MeCN containing 5 mM DBD-PZ and 70 mM diethylphosphorocyanidate, let stand at room temperature for 6 h, inject a 1 μ L 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, wash three times with 20 mL ethyl acetate, discard ethyl acetate washes, 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 DBD-PZ as orange crystals, mp 121–2°.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Inertsil ODS-2
Mobile phase: MeCN:water 65:35
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 10
Limit of detection: 3.9 fmol

OTHER SUBSTANCES

Simultaneous: indomethacin

KEY WORDS

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: Inject a 50 μ L aliquot of a solution in mobile phase.

HPLC VARIABLES**Column:** 100 × 4.6 5 μm Spheri-5 RP-8**Mobile phase:** MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na₂HPO₄ and 7 mM KH₂PO₄ to achieve pH 7.)**Flow rate:** 1**Injection volume:** 50**Detector:** F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 μg/mL reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetoneitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α-(3,4-dimethoxyphenyl)-4'-methylcinnamitrile. Dissolve 20 mmoles α-(3,4-dimethoxyphenyl)-4'-methylcinnamitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h, cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM**Retention time:** k' 4.1240**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES**Simultaneous:** ketoprofen, mefenamic acid, naproxen, probenecid, salicylic acid, valproic acid

KEY WORDS

post-column extraction

REFERENCEKim, M.; Stewart, J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α-phenylcinnamitrile quaternary ammonium salt as a new fluorescent ion-pair reagent. *J.Liq.Chromatogr.*, **1990**, *13*, 213-237

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: 4

OTHER SUBSTANCES

Simultaneous: aspirin, carprofen, diflunisal, fenbufen, indomethacin, naproxen, tolmetin

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: 100 μ L Urine + 200 μ L pH 5 citrate/NaOH buffer + 500 ng clofibrac acid + 5 mL diethyl ether:dichloromethane 80:20, shake for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness, add 200 μ L toluene and evaporate it to remove traces of water. Reconstitute the residue in 500 μ L dichloromethane, add 50 μ L 1 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 50 μ L 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 50 μ L 1 mg/mL FLOPA in dichloromethane, vortex, let stand at room temperature for 2 h, evaporate to dryness, reconstitute in mobile phase, inject a 10–20 μ L aliquot. (To hydrolyze glucuronides add 100 μ L 1 M NaOH to 100 μ L urine, let stand for 1 h, add 100 μ L 1 M HCl, proceed as above.) [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 0.6 mmoles 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 Åmolecular 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.]

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax Sil
Mobile phase: n-Hexane:chloroform:EtOH 100:10:1.25
Flow rate: 2
Injection volume: 10–20
Detector: F ex 305 em 355

CHROMATOGRAM

Retention time: 6.5 (R(-)), 13.5 (R(+))
Internal standard: clofibrac acid (5)

KEY WORDS

pharmacokinetics; chiral; derivatization; normal phase

REFERENCE

Spahn, H.; Langguth, P. Chiral amines derived from 2-arylpropionic acids: novel reagents for the liquid chromatographic (LC) fluorescence assay of optically active carboxylic acid xenobiotics. *Pharm.Res.*, **1990**, *7*, 1262–1268

SAMPLE**Matrix:** urine**Sample preparation:** Dilute urine 20-fold with 100 mM pH 2.0 phosphate buffer, extract twice with two volumes of diethyl ether, 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 80:20 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 278

CHROMATOGRAM**Retention time:** k' 5.0

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

SAMPLE**Matrix:** urine**Sample preparation:** 1 mL Urine + 100 μL 1 mg/mL methylprednisolone + 1 mL 1.5 M HCl + 500 μL water + 10 mL dichloromethane, shake 20 min, centrifuge at 250 g for 3 min. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute with 200 μL MeOH, inject 10-30 μL aliquot. (This assay determines free drug and metabolites. To determine total drug and metabolites (free plus conjugated) add 500 μL 1 M NaOH to 1 mL urine, let stand for 20 min at room temperature then proceed as above.)

HPLC VARIABLES**Column:** 250 × 4.5 Partisil 10 ODS-3**Mobile phase:** Gradient. A was MeCN:water 28:72 containing 0.05% phosphoric acid and 0.05% acetone. B was MeCN:50 mM KH₂PO₄ 50:50. A:B 100:0 for 8 min then to 0:100 over 6 min**Flow rate:** 2**Injection volume:** 10-30**Detector:** UV 220

CHROMATOGRAM**Retention time:** 21.8**Internal standard:** methylprednisolone (13.6)**Limit of quantitation:** 3 μg/mL

OTHER SUBSTANCES**Extracted:** metabolites

REFERENCE

Lockwood, G.F.; Wagner, J.G. High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids. *J.Chromatogr.*, **1982**, *232*, 335–343

ANNOTATED BIBLIOGRAPHY

Baeyens, W.R.G.; Van Der Weken, G.; Van Overbeke, A.; Zhang, X.R. A comparative study for the determination of ibuprofen in pharmaceutical preparations using different internal column diameters. *Biomed.Chromatogr.*, **1995**, *9*, 259–260

Haginaka, J.; Kanasugi, N. Enantioselectivity of bovine serum albumin-bonded columns produced with isolated protein fragments. *J.Chromatogr.A*, **1995**, *694*, 71–80 [chiral; also benzoin, clorazepate, fenopropfen, flurbiprofen, ibuprofen, ketoprofen, lorazepam, lormetazepam, oxazepam, pranoprofen, temazepam, warfarin]

Nakamura, K.; Fujima, H.; Kitagawa, H.; Wada, H.; Makino, K. Preparation and chromatographic characteristics of a chiral-recognizing perphenylated cyclodextrin column. *J.Chromatogr.A*, **1995**, *694*, 111–118 [chiral; also acetylpheneturide, alprenolol, arotinolol, atenolol, benzoin, biperiden, bunitrolol, chlormezanone, chlorphenesin, chlorpheniramine, eperisone, flavanone, oxprenolol, phenylethyl alcohol, phenylethylamine, pindolol, proglumide, propranolol, trihexyphenidyl]

Terfloth, G.J.; Pirkle, W.H.; Lynam, K.G.; Nicolas, E.C. Broadly applicable polysiloxane-based chiral stationary phase for high-performance liquid chromatography and supercritical fluid chromatography. *J.Chromatogr.A*, **1995**, *705*, 185–194 [chiral; HPLC; SFC; also carprofen, cicloprofen, etodolac, fenoprofen, flurbiprofen, naproxen, piroprofen, warfarin]

Naidong, W.; Lee, J.W. Development and validation of a liquid chromatographic method for the quantitation of ibuprofen enantiomers in human plasma. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 551–556 [chiral; plasma; LOQ 1 $\mu\text{g/mL}$; column temp 20]

Shirley, M.A.; Guan, X.; Kaiser, D.G.; Halstead, G.W.; Baillie, T.A. Taurine conjugation of ibuprofen in humans and in rat liver in vitro. Relationship to metabolic chiral inversion. *J.Pharmacol.Exp.Ther.*, **1994**, *269*, 1166–1175 [human; urine; gradient; extracted metabolites; microsomal incubations; rat; liver]

Van den Mooter, G.; Samyn, C.; Kinget, R. The relation between swelling properties and enzymatic degradation of azo polymers designed for colon-specific drug delivery. *Pharm.Res.*, **1994**, *11*, 1737–1741 [naproxen (IS); rat; cecal content release medium]

Van Overbeke, A.; Baeyens, W.; Van den Bossche, W.; Dewaele, C. Enantiomeric separation of amide derivatives of some 2-arylpropionic acids by HPLC on a cellulose-based chiral stationary phase. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 911–916 [chiral; derivatization; also, flurbiprofen, ketoprofen, tiaprofenic acid]

Jung, E.S.; Lee, H.S.; Rho, J.K.; Kwon, K.I. Simultaneous determination of ibuprofen and ibuprofen in human plasma by HPLC with column switching. *Chromatographia*, **1993**, *37*, 618–621 [plasma; column-switching; human; rat; pharmacokinetics; extracted metabolites, ibuprofen; N-phenylanthranilic acid (IS); LOD 100 ng/mL]

Szász, G.; Budvári-Bárány, Z.; Löre, A.; Radecky, G.; Shalaby, A. HPLC of antiphlogistic acids on silica dynamically modified with cetylpyridinium chloride. *J.Liq.Chromatogr.*, **1993**, *16*, 2335–2345 [also diclofenac, fenoprofen, ketoprofen, naproxen, nicotinic acid, niflumic acid, salicylic acid]

Pirkle, W.H.; Welch, C.J. An improved chiral stationary phase for the chromatographic separation of underivatized naproxen enantiomers. *J.Liq.Chromatogr.*, **1992**, *15*, 1947–1955 [chiral; also cicloprofen, fenoprofen, flurbiprofen, ketoprofen, naproxen, piroprofen, tiaprofenic acid]

Nahata, M.C. Determination of ibuprofen in human plasma by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1991**, *14*, 187–192 [plasma; LOD 250 ng/mL; isobutylphenyl acetate (IS)]

George, R.D.; Contario, J.J. Quantitation of terfenadine, pseudoephedrine hydrochloride, and ibuprofen in a liquid animal dosing formulation using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1988**, *11*, 475–488 [simultaneous degradation products, pseudoephedrine, terfenadine; formulations; stability-indicating; ion-exchange]

Karnes, H.T.; Rajasekharaiah, K.; Small, R.E.; Farthing, D. Automated solid phase extraction and HPLC analysis of ibuprofen in plasma. *J.Liq.Chromatogr.*, **1988**, *11*, 489–499 [plasma; SPE; flurbiprofen (IS); fluorescence detection]

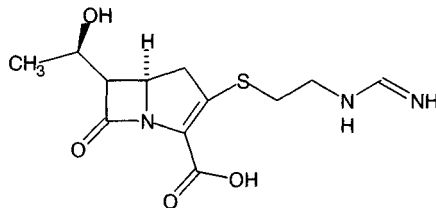
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- Hermansson, J.; Erikson, M. Direct liquid chromatographic resolution of acidic drugs using a chiral α 1-acid glycoprotein column (Enantiopac). *J.Liq.Chromatogr.*, **1986**, *9*, 621–639 [chiral; also bendroflumethiazide, disopyramide, ethotoin, hexobarbital, ketoprofen, naproxen, 2-phenoxypropionic acid, RAC 109]
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- Litowitz, H.; Olanoff, L.; Hoppel, C.L. Determination of ibuprofen in human plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1984**, *311*, 443–448
- Greenblatt, D.J.; Arendt, R.M.; Locniskar, A. Ibuprofen pharmacokinetics: use of liquid chromatography with radial compression separation. *Arzneimittelforschung*, **1983**, *33*, 1671–1673
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- Shimek, J.L.; Rao, N.G.; Khalil, S.K. High-pressure liquid chromatographic determination of ibuprofen in plasma. *J.Pharm.Sci.*, **1981**, *70*, 514–516
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Imipenem

Molecular formula: C₁₂H₁₇N₃O₄S

Molecular weight: 317.4

CAS Registry No.: 64221-86-9, 74431-23-5 (monohydrate)



SAMPLE

Matrix: aqueous humor, blood

Sample preparation: 1 mL Plasma or aqueous humor + 1 mL solvent, remove 0.5 mL mixture and add it to 0.5 mL MeOH, mix for 15 min, centrifuge at 4° at 4000 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 37-53 µm Pellicular ODS

Column: 250 × 4.6 Viosfer octadecyl (Violet)

Mobile phase: MeOH:100 mM pH 7.2 borate buffer 10:90

Flow rate: 1.5

Injection volume: 50

Detector: UV 313

CHROMATOGRAM

Retention time: 5

Limit of detection: 400 (plasma), 150 (aqueous humor) ng/mL

KEY WORDS

plasma

REFERENCE

Carlucci, G.; Biordi, L.; Bologna, M. Human plasma and aqueous humor determination of imipenem by liquid chromatography with ultraviolet detection. *J.Liq.Chromatogr.*, **1993**, *16*, 2347–2358

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. Pleural. Dilute human pleural samples with buffer, centrifuge, inject a 20 µL aliquot. (Buffer was 66.6 mM K₂HPO₄ adjusted to pH 7.40 with KH₂PO₄.)

HPLC VARIABLES

Column: 200 × 4.5 µm Nucleosil C18

Mobile phase: 15 mM Phosphoric acid adjusted to pH 7.0 with tetrabutylammonium hydroxide

Flow rate: 1

Injection volume: 20-100

Detector: UV 313

CHROMATOGRAM

Retention time: 4

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

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: blood, urine

Sample preparation: Add a stabilizing solution of 4-morpholineethanesulfonic acid buffer:ethylene glycol 1:1, prepare ultrafiltrate using an Amicon Centrifree micropartition system in a centrifuge, inject an aliquot.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeOH:100 mM sodium acetate 2:98, pH adjusted to 6.0 with acetic acid

Flow rate: 1

Detector: UV 298

CHROMATOGRAM

Retention time: 7.4

Limit of detection: 600 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 ceftiprome, ceftazidime, ceftriaxone, imipenem, and ciprofloxacin. *Antimicrob.Agents Chemother.*, **1992**, *36*, 2085-2092

SAMPLE

Matrix: enzyme incubations

Sample preparation: Add 2 volumes of MeOH, mix well, centrifuge at 3000 g for 15 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: TSKgel ODS-80Tm (Tosoh)

Mobile phase: MeOH:100 mM pH 7.0 phosphate buffer 4:100

Flow rate: 0.75

Injection volume: 20

Detector: UV 290

CHROMATOGRAM

Limit of quantitation: 1 μ g/mL

REFERENCE

Hikida, M.; Kawashima, K.; Yoshida, M.; Mitsuhashi, S. Inactivation of new carbapenem antibiotics by dehydropeptidase-I from porcine and human renal cortex. *J.Antimicrob.Chemother.*, **1992**, *30*, 129-134

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute 1:100 with mobile phase, inject a 30 μ L aliquot.

HPLC VARIABLES**Column:** 200 \times 4.6 RP-8 (Hewlett-Packard)**Mobile phase:** MeCN:MeOH:buffer 0.4:0.5:99.1, adjusted to pH 7.00 with NaOH (Buffer was 4 mM 3-[N-morpholino]propanesulfonic acid (MOPS) containing 2 g/L sodium hexane sulfate.)**Flow rate:** 1.8**Injection volume:** 30**Detector:** UV 250

CHROMATOGRAM**Retention time:** 5.5

OTHER SUBSTANCES**Extracted:** cilastatin

KEY WORDS

injections; total parenteral nutrition; stability-indicating

REFERENCEZaccardelli, D.S.; Krcmarik, C.S.; Wolk, R.; Khalidi, N. Stability of imipenem and cilastatin sodium in total parenteral nutrient solution. *J.Parenter.Enteral.Nutr.*, **1990**, *14*, 306–309

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 10 μ L aliquot of a 400 μ g/mL solution.

HPLC VARIABLES**Column:** 150 \times 4.6 Microsorb C8 80-315**Mobile phase:** 1 mM KH_2PO_4 adjusted to pH 6.8 with 500 mM NaOH**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV 300

REFERENCEConnolly, M.; Debenedetti, P.G.; Tung, H.-H. Freeze crystallization of imipenem. *J.Pharm.Sci.*, **1996**, *85*, 174–177

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 2.5-5 μ g/mL solution, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 80 \times 4.6 3.65 μ m Zorbax Rx-SIL (similar to Zorbax SB-C8 (Mac-Mod Analytical))**Mobile phase:** MeCN:buffer 0.5:99.5 (Buffer was 0.1% acetic acid adjusted to pH 7 with ammonium hydroxide.)**Flow rate:** 1**Injection volume:** 20**Detector:** UV 296

CHROMATOGRAM**Retention time:** k' 3.5

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

HPLC VARIABLES

Column: 150 × 4.6 PLRP-S styrene-divinylbenzene copolymer (Polymer Labs)

Mobile phase: Gradient. MeCN:20 mM pH 7.2 KH₂PO₄-NaOH buffer 3:97 to 7:93 in 22 min.

Column temperature: 40

Flow rate: 1.2

Detector: UV 295

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

pH >4 buffer

REFERENCE

Smith, G.B.; Dezeny, G.C.; Douglas, A.W. Stability and kinetics of degradation of imipenem in aqueous solution. *J.Pharm.Sci.*, **1990**, 79, 732-740

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Partisil PXS 5/25 PAC

Mobile phase: Gradient. MeCN:water from 25:75 to 50:50 in 30 min.

Flow rate: 2

Detector: UV 320

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

pH 4 buffer

REFERENCE

Smith, G.B.; Dezeny, G.C.; Douglas, A.W. Stability and kinetics of degradation of imipenem in aqueous solution. *J.Pharm.Sci.*, **1990**, 79, 732-740

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 20:80

Flow rate: 2

Injection volume: 25

Detector: UV 300

OTHER SUBSTANCES

Also analyzed: penicillin G (UV 227)

REFERENCE

Eley, A.; Greenwood, D. Beta-lactamases of type culture strains of the *Bacteroides fragilis* group and of strains that hydrolyse cefoxitin, latamoxef and imipenem. *J.Med.Microbiol.*, **1986**, *21*, 49–57

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Jenke, D.R. Drug binding by reservoirs in elastomeric infusion devices. *Pharm.Res.*, **1994**, *11*, 984–989 [formulations; saline; 5% dextrose; simultaneous cilastatin]

Ebey, W.J.; Boucher, B.A.; Pieper, J.A. A rapid HPLC method for determination of imipenem in plasma. *J.Liq.Chromatogr.*, **1988**, *11*, 3471–3481 [plasma; LOD 1000 ng/mL]

Krausse, R.; Ullmann, U. Determination of imipenem and cilastatin in serum and tissue by high-pressure liquid chromatography. *Infection*, **1986**, *14*, 243–245

Gravallese, D.A.; Musson, D.G.; Pauliukonis, L.T.; Bayne, W.F. Determination of imipenem (N-formimidoyl thienamycin) in human plasma and urine by high-performance liquid chromatography, comparison with microbiological methodology and stability. *J.Chromatogr.*, **1984**, *310*, 71–84 [plasma; urine; serotonin (IS); ultrafiltrate; LOD 300 ng/mL (plasma); LOD 1 μg/mL (urine); non-interfering cilastatin; pharmacokinetics]

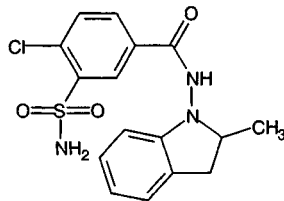
Myers, C.M.; Blumer, J.L. Determination of imipenem and cilastatin in serum by high-pressure liquid chromatography. *Antimicrob.Agents Chemother.*, **1984**, *26*, 78–81

Indapamide

Molecular formula: C₁₆H₁₆ClN₃O₃S

Molecular weight: 365.8

CAS Registry No.: 26807-65-8



SAMPLE

Matrix: blood

Sample preparation: 1 mL Whole blood + 1 mL 4 µg/mL glipizide in 50 mM pH 6.6 KH₂PO₄, vortex, add 6 mL diethyl ether, shake at 150 ± 20 oscillations/min on a reciprocating shaker for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness at 37° under a stream of nitrogen. Dissolve residue in 200 µL mobile phase, centrifuge at 1000 g for 3 min, inject a 40 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Nucleosil C18

Mobile phase: MeCN:isopropanol:buffer 30:5:65 (Buffer was 80 mM ammonium acetate adjusted to pH 3.5 with concentrated HCl.)

Flow rate: 1

Injection volume: 40

Detector: UV 241

CHROMATOGRAM

Retention time: 5.2

Internal standard: glipizide (5.9)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, aspirin, caffeine, dextromethorphan, ibuprofen, nicotine, phenylpropranolamine, theophylline

KEY WORDS

whole blood

REFERENCE

Miller, R.B.; Dadgar, D.; Lalande, M. High-performance liquid chromatographic method for the determination of indapamide in human whole blood. *J.Chromatogr.*, **1993**, 614, 293-298

SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL 10 mM HCl. 2 mL Plasma + 2 mL 2 µg/mL IS in water + 10 mL 10 mM HCl, add to the SPE cartridge, wash with 4 mL 10 mM HCl, elute with 1 mL MeOH, inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

Column: 250 × 4 10 µm LiChrosorb SI 60 ODS

Mobile phase: MeCN:10 mM pH 3.5 sodium phosphate 30:70

Flow rate: 1

Injection volume: 20

Detector: UV 241

CHROMATOGRAM

Retention time: 9

Internal standard: sulfadimethoxime (6.2)

Limit of detection: 10 ng/mL

KEY WORDS

plasma; SPE; see Anal.Abs. 1986, 48, 12D80

REFERENCE

Gaetani, E.; Laureri, C.F.; Vitto, M.; Borghi, L.; Elia, G.F.; Novarini, A. Determinazione dell'indapamide nel plasma mediante HPLC [Determination of indapamide in plasma by HPLC]. *Boll.Chim.Farm.*, 1986, 125, 35-37

SAMPLE

Matrix: blood

Sample preparation: Keep tubes in crushed ice except when being processed throughout this procedure. 2 mL Plasma + 100 μ L 10 μ g/mL sulfanilamide in MeCN + 8 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Remove ether layer and add it to 1 mL 100 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 1 mL 100 mM HCl and 500 μ L 50 mM pH 7.4 sodium phosphate, add 8 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μ L mobile phase, inject 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax ODS

Mobile phase: MeCN:100 mM pH 3.6 sodium acetate buffer 43:57

Column temperature: 54

Flow rate: 1

Injection volume: 50

Detector: UV 241

CHROMATOGRAM

Retention time: 6.3

Internal standard: sulfanilamide (5.3)

Limit of detection: 25 ng/mL

KEY WORDS

plasma

REFERENCE

Choi, R.L.; Rosenberg, M.; Grebow, P.E.; Huntley, T.E. High-performance liquid chromatographic analysis of indapamide (RHC 2555) in urine, plasma and blood. *J.Chromatogr.*, 1982, 230, 181-187

SAMPLE

Matrix: bulk, formulations, urine

Sample preparation: Bulk, tablets. Weigh out amount containing 10 mg indapamide, dissolve in 10 mL 150 μ g/mL sulfisoxazole in MeOH, make up to 100 mL with MeOH, inject an aliquot. Urine. 1 mL Urine + 1 mL 150 μ g/mL sulfisoxazole in MeOH + 10 mL ethyl acetate, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 10 μ L aliquot. (If indapamide concentration is >100 ng/mL mix 1 mL urine, 1 mL 150 μ g/mL sulfisoxazole in MeOH, and 8 mL mobile phase, vortex, inject a 10 μ L aliquot.)

HPLC VARIABLES

Guard column: 20 \times 4 Bondapak C18 Corasil

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeCN:buffer 35:65 (Buffer was water adjusted to pH 2.8 with 10% phosphoric acid.)

Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7
Internal standard: sulfisoxazole (4)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

tablets

REFERENCE

Pietta, P.; Calatroni, A.; Rava, A. High-performance liquid chromatographic assay for monitoring indapamide and its major metabolite in urine. *J.Chromatogr.*, **1982**, *228*, 377-381

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Chiralcel OD-R
Mobile phase: MeCN:water 40:60
Column temperature: 40
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 11.3, 15.8 (enantiomers)

KEY WORDS

chiral

REFERENCE

Application Guide for Chiral Column Selection, Second Edition, Chiral Technologies Inc., Exton PA, 1995, p. 39

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 58:35:7 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 248

KEY WORDS

chiral; $\alpha = 1.08$

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 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Lichrosorb RP-18**Mobile phase:** MeOH:buffer 50:50 (Buffer was 1% aqueous acetic acid containing 0.2% triethylamine.)**Flow rate:** 1**Injection volume:** 20**Detector:** UV 250

CHROMATOGRAM**Retention time:** 11**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Simultaneous:** degradation products

KEY WORDS

stability-indicating

REFERENCEPadval, M.V.; Bhargava, H.N. Liquid chromatographic determination of indapamide in the presence of its degradation products. *J.Pharm.Biomed.Anal.*, **1993**, *11*, 1033–1036

ANNOTATED BIBLIOGRAPHYChen, D. [Determination of indapamide in human serum by high performance liquid chromatography]. *Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao*, **1990**, *12*, 286–289

Insulin

Molecular formula: $C_{258}H_{383}N_{65}O_{77}S_6$ (human)

Molecular weight: 5807.6 (human)

CAS Registry No.: 9004-10-8 (injection), 8049-62-5 (zinc suspension), 11061-68-0 (human), 12584-58-6 (pig), 11070-73-8 (cow), 9004-14-2 (neutral insulin), 8049-62-5 (isophane insulin), 9004-17-5 (protamine zinc suspension)

SAMPLE

Matrix: blood, tissue

Sample preparation: Pancreas. 1 g Tissue + 25 mL 6% trichloroacetic acid, homogenize, centrifuge, wash with 1 mL 5% trichloroacetic acid, extract precipitate twice at 37° by shaking for 2 h with 4 mL acid ethanol (A), adjust pH to 8.5-9 with concentrated ammonium hydroxide, inject 200 μ L aliquot. (Acid ethanol (A) was 15 mL EtOH + 5 mL water + 3 mL concentrated HCl.) Plasma. 1 mL Plasma + 2 mL water + 7.5 mL cold acid ethanol (B), stand at 4° for 12 h, centrifuge at 2800 rpm at 4° for 20 min, adjust pH of supernatant to 8.3 with concentrated ammonium hydroxide, keep at 4° for 15 min, centrifuge at 2800 rpm at 4° for 20 min, adjust pH of supernatant to 5.3 with 4 M HCl, for each 1 mL add 25 μ L 2 M ammonium acetate, readjust pH to 5.3, to each 10 mL slowly add 15 mL cold EtOH and 25 mL diethyl ether, keep at 4° for 12 h, centrifuge at 2800 rpm at 4° for 30 min. Remove precipitate and dry it under nitrogen gas. Dissolve in 100 mM pH 3.10 NaH_2PO_4 , inject aliquot. (Acid ethanol (B) was 375 mL 95% EtOH and 7.5 mL concentrated HCl.)

HPLC VARIABLES

Column: 250 \times 4.5 Spherisorb S5 ODS2

Mobile phase: Gradient. A was MeCN. B was 100 mM pH 3.10 NaH_2PO_4 . A:B from 0:100 to 28:72 over 14 min, to 28.8:71.2 over 8 min, to 29.2:71.8 over 10 min, to 39.2:61.8 over 5 min, to 60:40 over 4 min.

Flow rate: 1

Injection volume: 200

Detector: UV 220

CHROMATOGRAM

Retention time: 33 (human), 35 (porcine)

KEY WORDS

pancreas; serum; human; pig

REFERENCE

Knip, M. Analysis of pancreatic peptide hormones by reversed-phase high-performance liquid chromatography. *Horm. Metab. Res.*, **1984**, *16*, 487-491

SAMPLE

Matrix: bulk

Sample preparation: Dissolve in mobile phase at 100-200 μ g/mL, inject 100 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 3 μ m Supelcosil LC-18DB ODS

Column: 150 \times 4.6 3 μ m Supelcosil LC-18DB ODS

Mobile phase: Gradient. A was 500 mM sodium sulfate + 300 mM NaH_2PO_4 adjusted to pH 2.5 with perchloric acid. B was water. C was MeCN:water 60:40. A:B:C at 20:30:50 for 3 min then to 20:22.5:57.5 over 27 min.

Column temperature: 45

Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 18 (bovine), 19.5 (bovine MDA), 21 (human), 22 (porcine), 23 (human MDA), 24 (porcine MDA)

KEY WORDS

cow; pig; human

REFERENCE

Janssen, P.S.L.; van Nispen, J.W.; van Zeeland, M.J.M.; Melgers, P.A.T.A. Complementary information from isotachopheresis and high-performance liquid chromatography in peptide analysis. *J.Chromatogr.*, **1989**, *470*, 171–183

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 10 mg microspheres in 2 mL MeCN, centrifuge at 3000 rpm for 5 min, discard MeCN, repeat process three times, dissolve pellet in 5 mL pH 7.4 Tris buffer containing 0.1% trifluoroacetic acid, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 6 Asahipak ODP-50 6D (Asahi Chemical)

Mobile phase: MeCN:buffer 28:72 (Buffer was 0.3% ethanolamine adjusted to pH 2.0 with phosphoric acid.)

Column temperature: 40

Flow rate: 2

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

microspheres; cow

REFERENCE

Uchida, T.; Yagi, A.; Oda, Y.; Nakada, Y.; Goto, S. Instability of bovine insulin in poly(lactide-co-glycolide) (PLGA). *Chem.Pharm.Bull.*, **1996**, *44*, 235–236

SAMPLE

Matrix: formulations

Sample preparation: 100 μ L Injection + 50 μ L 50 μ g/mL benzoic acid in water + 250 μ L water, vortex briefly, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 300 \AA silica for proteins and peptides (Vydac)

Mobile phase: MeCN:50 mM pH 2.4 KH_2PO_4 , 25:75

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM**Retention time:** 11.16**Internal standard:** benzoic acid

OTHER SUBSTANCES**Simultaneous:** degradation products

KEY WORDSinjections; stability-indicating

REFERENCE

Hoyer, G.L.; Nolan, P.E., Jr.; LeDoux, J.H.; Moore, L.A. Selective stability-indicating high-performance liquid chromatographic assay for recombinant human regular insulin. *J.Chromatogr.A*, **1995**, 699, 383–388

SAMPLE**Matrix:** formulations**Sample preparation:** Prepare a 150 µg/mL solution in 10 mM HCl, inject a 20 µL aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 µm Ultremex octadecylsilane**Mobile phase:** MeCN:buffer 26:74 (Buffer was 200 mM sodium sulfate adjusted to pH 2.3.)**Column temperature:** 40**Flow rate:** 0.9**Injection volume:** 20**Detector:** UV 214

CHROMATOGRAM**Retention time:** 17.5

KEY WORDSinjections

REFERENCE

Lookabaugh, M.; Biswas, M.; Krull, I.S. Quantitation of insulin injection by high-performance liquid chromatography and high-performance capillary electrophoresis. *J.Chromatogr.*, **1991**, 549, 357–366

SAMPLE**Matrix:** formulations

HPLC VARIABLES**Column:** 50 × 4.6 C4 wide pore (300 Å) (Supelco)**Mobile phase:** Gradient. A was MeOH:isopropanol:water 4:1:95 containing 2.8 g/L NaCl. B was MeOH:isopropanol:water 60:10:30 containing 4.2 g/L NaCl. A:B from 45:55 to 30:70 over 5 min.**Flow rate:** 1**Injection volume:** 20**Detector:** E, Bioanalytical systems Model LC-4B, dual glassy-carbon working electrode used in parallel mode, +0.65 V and +0.80 V (monitored), stainless steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed at 0–5° through a 2 mL knitted coil of 0.5 mm i.d. PTFE tubing irradiated with a low pressure mercury lamp (Photronix Model 816) to the detector.

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES

Also analyzed: b-lactoglobulin A, lysozyme, phenylalanine, ribonuclease A, tryptophan, tyramine

KEY WORDS

post-column reaction

REFERENCE

Dou, L.; Krull, I.S. Determination of aromatic and sulfur-containing amino acids, peptides, and proteins using high-performance liquid chromatography with photolytic electrochemical detection. *Anal.Chem.*, **1990**, *62*, 2599-2606

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Synchropak C4

Mobile phase: Gradient. A was 0.05% trifluoroacetic acid in water. B was 0.05% trifluoroacetic acid in MeCN. A:B from 74:26 to 38:62 over 15 min.

Flow rate: 1.5

Detector: UV 220

CHROMATOGRAM

Retention time: 4.7

REFERENCE

Ho, H.-O.; Hsiao, C.-C.; Sheu, M.-T. Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs. *J.Pharm.Sci.*, **1996**, *85*, 138-143

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: 10

OTHER SUBSTANCES

Simultaneous: angiotensin II, carbonic anhydrase, cytochrome C, leucine enkephalin, lysozyme, myoglobin, RNase

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: solutions

HPLC VARIABLES

Column: 150 × 4.6 Develosil ODS-HG-5 (Nomura Chemical)
Mobile phase: MeCN:100 mM pH 9.0 phosphate buffer 26:74
Column temperature: 40
Flow rate: 0.8
Detector: UV 214

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Yomota, C.; Yoshii, Y.; Takahata, T.; Okada, S. Separation of B-3 monodesamidoinsulin from human insulin by high-performance liquid chromatography under alkaline conditions. *J.Chromatogr.A*, **1996**, 721, 89–96

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Protein & Peptide C18 (Vydac)
Mobile phase: MeCN:buffer 26:74 (Buffer was 28.4 g sodium sulfate and 2.7 mL phosphoric acid in 1 L water, pH adjusted to 2.3 with ethanolamine (if necessary).)
Column temperature: 40
Flow rate: 0.8
Detector: UV 214

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Yomota, C.; Yoshii, Y.; Takahata, T.; Okada, S. Separation of B-3 monodesamidoinsulin from human insulin by high-performance liquid chromatography under alkaline conditions. *J.Chromatogr.A*, **1996**, 721, 89–96

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4 Armsorb-Si-300 p (DM) (Armchrom, Yerevan, Armenia)
Mobile phase: Gradient. A was MeCN:1 M ammonium acetate 10:90. B was MeCN:1 M ammonium acetate 50:50. A:B from 76:24 to 66:34 over 40 min.
Flow rate: 0.8
Detector: UV 200

CHROMATOGRAM

Retention time: 26

OTHER SUBSTANCES

Simultaneous: proinsulin

KEY WORDS

recombinant; comparison with capillary electrophoresis

REFERENCE

Klyushnichenko, V.E.; Koulich, D.M.; Yakimov, S.A.; Maltsev, K.V.; Grishina, G.A.; Nazimov, I.V.; Wulfson, A.N. Recombinant human insulin. III. High-performance liquid chromatography and high-performance capillary electrophoresis control in the analysis of step-by-step production of recombinant human insulin. *J.Chromatogr.A*, **1994**, *661*, 83–92

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 600 × 7.5 TSK G 2000 SW (TOSOH)

Mobile phase: MeCN:buffer 5:95 (Buffer was 100 mM pH 7.0 phosphate buffer containing 200 mM sodium sulfate.)

Flow rate: 1

Detector: UV 200

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Simultaneous: proinsulin

KEY WORDS

recombinant; comparison with capillary electrophoresis; SEC

REFERENCE

Klyushnichenko, V.E.; Koulich, D.M.; Yakimov, S.A.; Maltsev, K.V.; Grishina, G.A.; Nazimov, I.V.; Wulfson, A.N. Recombinant human insulin. III. High-performance liquid chromatography and high-performance capillary electrophoresis control in the analysis of step-by-step production of recombinant human insulin. *J.Chromatogr.A*, **1994**, *661*, 83–92

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1 mg/mL solution in MeCN:water 20:80, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: Nucleosil 100-5 C18 RP

Mobile phase: Gradient. MeCN:buffer from 20:80:40:60 over 20 min. (Buffer was 83 mM phosphoric acid adjusted to pH 2.25 with triethylamine.)

Injection volume: 10

Detector: UV 215

CHROMATOGRAM

Retention time: 15

REFERENCE

Lenz, V.J.; Gattner, H.-G.; Leithäuser, M.; Brandenburg, D.; Wollmer, A.; Höcker, H. Proteolyses of a fluorogenic insulin derivative and native insulin in reversed micelles monitored by fluorescence emission, reversed-phase high-performance liquid chromatography, and capillary zone electrophoresis. *Anal.Biochem.*, **1994**, *221*, 85–93

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:** 10.7

OTHER SUBSTANCES**Simultaneous:** angiotensin I, angiotensin II, bradykinin, leucin enkephalin, lysozyme, melittin, methionine enkephalin, oxytocin

REFERENCE*Bodman Product Guide, Bodman, Aston PA, 1992, p. 104*

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 × 4.1 10 μm PRP-3 (Hamilton)**Mobile phase:** Gradient. A was 0.1% trifluoroacetic acid in 50 mM NaOH. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 40:60 over 30 min.**Flow rate:** 2**Detector:** UV 220

CHROMATOGRAM**Retention time:** 14

OTHER SUBSTANCES**Simultaneous:** cytochrome C, lysozyme, myoglobin, ribonuclease A, trypsin

REFERENCE*Rainin Catalog 1991-2, Rainin Instrument Co., Woburn MA, 1991, p. 3.33*

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 PLRP-S 1000Å (Polymer Labs)**Mobile phase:** Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in 95% MeCN. A:B from 80:20 to 40:60 over 22 min.**Flow rate:** 1.5**Detector:** UV 220

CHROMATOGRAM**Retention time:** 5

OTHER SUBSTANCES

Simultaneous: bovine serum albumin, cytochrome C, lysozyme, myoglobin, ovalbumin, ribonuclease

REFERENCE

Rainin Catalog 1991-2, Rainin Instrument Co., Woburn MA, 1991, p. 3.63

SAMPLE

Matrix: solutions

Sample preparation: Dissolve 70 mg trinitrobenzenesulfonic acid in 1 mL 100 mM pH 8.2 sodium bicarbonate and immediately add an aliquot to 50 volumes of 10 mg/mL insulin in 100 mM pH 8.2 sodium bicarbonate, let stand in the dark at room temperature for 2 h, add to a 150 × 60 column of Sephadex G25 made up in 100 mM pH 8.2 sodium bicarbonate, collect the major colored band and lyophilize it. Reconstitute, inject an aliquot.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: Gradient. MeCN:100 mM pH 3.6 sodium phosphate 25:75 for 10 min, to 45:55 over 1 h

Flow rate: 1

Detector: UV 280

CHROMATOGRAM

Retention time: 50

KEY WORDS

derivatization

REFERENCE

Wallace, G.R.; McLeod, A.; Chain, B.M. Chromatographic analysis of the trinitrophenyl derivatives of insulin. *J.Chromatogr.*, **1988**, *427*, 239–246

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Lakhiari, H.; Legendre, E.; Muller, D.; Jozefonvicz, J. High-performance affinity chromatography of insulin on coated silica grafted with sialic acid. *J.Chromatogr.B*, **1995**, *664*, 163–173

Calvaruso, G.; Tesoriere, G.; Vento, R.; Giuliano, M.; Carabillò, M. High-performance liquid chromatographic method for the determination of insulin synthesis in biological systems. *J.Chromatogr.B*, **1994**, *660*, 259–264 [SEC; GPC; reverse phase]

Dimov, N.; Simeonov, S. Experimental models for optimization of insulin separation on reversed phase columns. *Biomed.Chromatogr.*, **1994**, *8*, 32–36 [gradient; cow; pig]

Kliushnichenko, V.E.; Iakimov, S.A.; Arutiunian, A.M.; Ivanov, A.E.; Mal'tsev, K.V.; Vul'fson, A.N. [Genetic engineering of human insulin. IV. Development and optimization of an analysis system using reversed phase high pressure liquid chromatography]. *Bioorg.Khim.*, **1994**, *20*, 1080–1088

Klyushnichenko, V.E.; Yakimov, S.A.; Arutyunyan, A.M.; Ivanov, A.E.; Maltsev, K.V.; Wulfson, A.N. Recombinant human insulin V. Optimization of the reversed-phase high-performance liquid chromatographic separation. *J.Chromatogr.B*, **1994**, *662*, 363–369 [gradient]

Ohkubo, T. High performance liquid chromatographic analysis of polypeptide hormones in transplanted rat islets. *Biomed.Chromatogr.*, **1994**, *8*, 301–305

Yamamoto, A.; Taniguchi, T.; Rikyuu, K.; Tsuji, T.; Fujita, T.; Murakami, M.; Muranishi, S. Effect of various protease inhibitors on the intestinal absorption and degradation of insulin in rats. *Pharm.Res.*, **1994**, *11*, 1496–1500 [rat; gradient]

Salem, I.I.; Bedmar, M.C.; Medina, M.M.; Cerezo, A. Insulin evaluation in pharmaceuticals: Variables in RP-HPLC and method validation. *J.Liq.Chromatogr.*, **1993**, *16*, 1183–1194 [formulations]

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- Cruz, N.; Antonio, S.; de Anda, R.; Gosset, G.; Bolivar, F. Preparative isolation by high performance liquid chromatography of human insulin B chain produced in *Escherichia coli*. *J.Liq.Chromatogr.*, **1990**, *13*, 1517–1528
- Caprioli, R.M.; DaGue, B.; Fan, T.; Moore, W.T. Microbore HPLC/mass spectrometry for the analysis of peptide mixtures using a continuous flow interface. *Biochem.Biophys.Res.Commun.*, **1987**, *146*, 291–299 [LC-MS; microbore; gradient; UV detection; cow; sheep; pig; horse]
- Schrader, E.; Pfeiffer, E.F. The influence of motion and temperature upon the aggregational behaviour of soluble insulin formulations investigated by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1985**, *8*, 1139–1157 [GPC; SEC; reverse-phase; pig; human]
- Schrader, E.; Pfeiffer, E.F. HPLC-gel filtration of insulin during short and long time infusion by artificial delivery systems. *J.Liq.Chromatogr.*, **1985**, *8*, 1121–1137 [GPC; SEC]
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- Ohta, M.; Tokunaga, H.; Kimura, T.; Satoh, H.; Kawamura, J. Analysis of insulins by high-performance liquid chromatography. III. Determination of insulin in various preparations. *Chem.Pharm.Bull.*, **1984**, *32*, 4641–4649
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- Ohta, M.; Tokunaga, H.; Kimura, T.; Satoh, H.; Kawamura, J. [Analysis of insulin in preparations by high performance liquid chromatography]. *Yakugaku Zasshi*, **1982**, *102*, 1092–1094
- Pocker, Y.; Biswas, S.B. A simple liquid chromatographic method for analysis of insulin and its derivatives. *J.Liq.Chromatogr.*, **1982**, *5*, 1–14 [SEC; GPC]

Interferon

Molecular formula: $C_{860}H_{1353}N_{229}O_{255}S_9$

Molecular weight: 19269.1

CAS Registry No.: 76543-88-9 (α A), 99210-65-8 (α 2B), 98059-61-1 (γ)

SAMPLE

Matrix: solutions

Sample preparation: Dilute PCR product solution 1:5 with water, inject an 80 μ L aliquot.

HPLC VARIABLES

Column: 35 \times 4.6 2.5 μ m TSK DEAE-NPR (Perkin-Elmer)

Mobile phase: Gradient. A was 25 mM pH 9.0 Tris-HCl buffer. B was 25 mM pH 9.0 Tris-HCl buffer containing 1 M NaCl. A:B from 70:30 to 45:55 over 30 s, to 35:65 over 2.5 min, to 0:100 over 30 s, maintain at 0:100 for 30 s, return to initial conditions over 30 s, re-equilibrate for 30 s.

Flow rate: 1

Injection volume: 80

Detector: UV 260

CHROMATOGRAM

Retention time: 2.2

REFERENCE

Zeillinger, R.; Schneeberger, C.; Speiser, P.; Kury, F. Rapid quantitative analysis of differential PCR products by high-performance liquid chromatography. *BioTechniques*, **1993**, *15*, 89–95

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a solution in glycerol:20 mM pH 7.2 sodium phosphate buffer 30:70.

HPLC VARIABLES

Column: 150 \times 3 Separon SGX C-18 glass column

Mobile phase: Gradient. A was 1 M pyridine adjusted to pH 5.0 with acetic acid. B was n-propanol. A:B 80:20 for 20 min, to 30:70 over 1 h.

Flow rate: 0.25

Detector: UV 280; bioassay

CHROMATOGRAM

Retention time: 46

REFERENCE

Aboagye-Mathiesen, G.; Toth, F.D.; Juhl, C.; Norskov-Lauritsen, N.; Petersen, P.M.; Ebbesen, P. Purification of human placental trophoblast interferon by two-dimensional high performance liquid chromatography. *Prep.Biochem.*, **1991**, *21*, 35–51

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 600 \times 7.8 10 μ m Protein Pak J125 (Waters)

Mobile phase: Propylene glycol:buffer 25:75 (Buffer was 20 mM pH 7.0 sodium phosphate buffer containing 500 mM sodium sulfate and 0.04% Tween 20.)

Flow rate: 0.5

Detector: UV 214

KEY WORDS

interferon-omega1; GPC

REFERENCE

Adolf, G.R.; Maurer-Fogy, I.; Kalsner, I.; Cantell, K. Purification and characterization of natural human interferon omega 1. Two alternative cleavage sites for the signal peptidase. *J.Biol.Chem.*, **1990**, *265*, 9290-9295

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Bakerbond WP C18

Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B 80:20 for 2 min, to 32:68 over 24 min, maintain at 32:68 for 10 min.

Column temperature: 30

Flow rate: 1

Detector: UV 214; UV 280

CHROMATOGRAM

Retention time: 22

KEY WORDS

interferon-omega1

REFERENCE

Adolf, G.R.; Maurer-Fogy, I.; Kalsner, I.; Cantell, K. Purification and characterization of natural human interferon omega 1. Two alternative cleavage sites for the signal peptidase. *J.Biol.Chem.*, **1990**, *265*, 9290-9295

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 4 Nucleosil 5C18

Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 35:65 to 55:45 over 40 min.

Flow rate: 0.9

Detector: UV 210

CHROMATOGRAM

Retention time: 20 (Mf-1), 22 (Mf-2), 24 (Ms)

REFERENCE

Nakagawa, S.; Honda, S.; Sugino, H.; Kusumoto, S.; Sasaoki, K.; Nishi, K.; Kakinuma, A. Characterization of three species of Escherichia coli-derived human leukocyte interferon A separated by reverse-phase high-performance liquid chromatography. *J.Interferon.Res.*, **1987**, *7*, 285-299

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Whatman Copell ODS

Column: 250 × 10 Synchronapak C18 RP-P

Mobile phase: Gradient. A was 0.025% trifluoroacetic acid in water. B was 0.025% trifluoroacetic acid in MeCN. A:B from 70:30 to 40:60 over 30 min.

Flow rate: 2

Detector: UV 220

CHROMATOGRAM

Retention time: 26 (IFN- α A)

OTHER SUBSTANCES

Simultaneous: other forms of interferon

REFERENCE

Felix, A.M.; Heimer, E.P.; Tarnowski, S.J. Analysis of different forms of recombinant human interferons by high-performance liquid chromatography. *Methods Enzymol.*, **1986**, *119*, 242–248

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Whatman Copell ODS

Column: 250 × 10 Synchronapak C18 RP-P

Mobile phase: Gradient. A was 0.025% trifluoroacetic acid in water. B was 0.025% trifluoroacetic acid in MeCN. A:B from 70:30 to 40:60 over 30 min.

Flow rate: 2

Detector: UV 220

CHROMATOGRAM

Retention time: 26 (IFN- α A)

REFERENCE

Felix, A.M.; Heimer, E.P.; Lambros, T.J.; Swistok, J.; Tarnowski, S.J.; Wang, C.-T. Analysis of different forms of recombinant human leukocyte interferons and synthetic fragments by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, *327*, 359–368

SAMPLE

Matrix: solutions

Sample preparation: Centrifuge at 5000 g at 4° for 10 min, inject an aliquot.

HPLC VARIABLES

Column: 50 × 5 10 μ m Mono-S HPLC cation-exchange column (Pharmacia)

Mobile phase: Gradient. A was 10 mM pH 7.0 sodium phosphate in ethylene glycol:water 20:80. B was 10 mM pH 7.0 sodium phosphate + 400 mM NaCl in ethylene glycol:water 20:80. A:B 100:0 for 30 min then to 0:100 over 60 min.

Flow rate: 0.5

Detector: bioassay

CHROMATOGRAM

Retention time: 70

KEY WORDS

crude mixtures

REFERENCE

Friedlander, J.; Fischer, D.G.; Rubinstein, M. Isolation of two discrete human interferon-gamma (immune) subtypes by high-performance liquid chromatography. *Anal.Biochem.*, **1984**, *137*, 115-119

SAMPLE

Matrix: solutions

Sample preparation: Pump 125 mL of a solution of interferon in ethylene glycol:50 mM sodium phosphate buffer containing 1 M NaCl 50:50 onto the column (which was previously equilibrated with 1 M NaCl in ethylene glycol:water 50:50) then start the gradient.

HPLC VARIABLES

Column: 300 × 4.6 10 μm Chromegabond octyl

Mobile phase: Gradient. A was pyridine:formic acid:isopropanol:n-butanol:water 8:8:20:3.3:60.7. B was pyridine:formic acid:isopropanol:n-butanol:water 8:8:25:20:39. A: B from 100:0 to 75:25 over 30 min, to 45:55 over 190 min, to 0:100 over 20 min, maintain at 0:100 for 1 h.

Flow rate: 0.45

Injection volume: 125000

Detector: F; bioassay

CHROMATOGRAM

Retention time: 100

REFERENCE

Friesen, H.J.; Stein, S.; Pestka, S. Purification of human fibroblast interferon by high-performance liquid chromatography. *Methods Enzymol.*, **1981**, *78*, 430-435

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: MN-cyanopropyl

Mobile phase: Gradient. Pyridine:formic acid:n-propanol:water from 8:8:0:84 to 8:8:40:44 over 1 h.

Flow rate: 0.3

Detector: F; bioassay

CHROMATOGRAM

Retention time: 40

REFERENCE

Friesen, H.J.; Stein, S.; Pestka, S. Purification of human fibroblast interferon by high-performance liquid chromatography. *Methods Enzymol.*, **1981**, *78*, 430-435

ANNOTATED BIBLIOGRAPHY

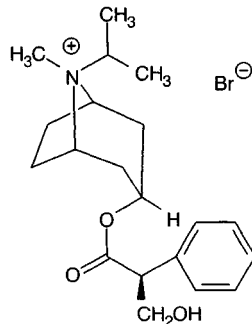
Feng, W.; Geng, X. Studies on silica-bonded monoclonal antibody packing material for separation of recombinant interferon by high performance immunoaffinity chromatography. *Biomed.Chromatogr.*, **1993**, *7*, 317-320 [column was anti-interferon monoclonal antibody bonded to silica; gradient]

Ipratropium Bromide

Molecular formula: C₂₀H₃₀BrNO₃

Molecular weight: 412.4

CAS Registry No.: 22254-24-6, 66985-17-9 (monohydrate)



SAMPLE

Matrix: formulations

Sample preparation: Dilute with water to a concentration of 125 µg/mL, add a 300 µL aliquot to 250 µL 2 mg/mL mepivacaine hydrochloride in water, vortex, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 µm NovaPak C18 radial compression

Mobile phase: Gradient. A was 2.5 mM PIC B-8 Low UV (Waters) in THF:water 40:60. B was water. C was MeOH:water 50:50. A:B:C 50:50:0 for 7.7 min, to 60:15:25 over 5.3 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 2

Injection volume: 10

Detector: UV 220

CHROMATOGRAM

Retention time: 5.9

Internal standard: mepivacaine (8.2)

OTHER SUBSTANCES

Simultaneous: albuterol, fenoterol, terbutaline

KEY WORDS

nebulizer solutions; stability-indicating

REFERENCE

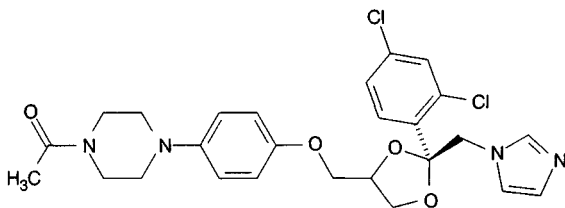
Jacobson, G.A.; Peterson, G.M. High-performance liquid chromatographic assay for the simultaneous determination of ipratropium bromide, fenoterol, salbutamol and terbutaline in nebulizer solution. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 825-832

Ketoconazole

Molecular formula: C₂₆H₂₆Cl₂N₄O₄

Molecular weight: 531.4

CAS Registry No.: 65277-42-1



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 2 μ g clotrimazole + hexane:isoamyl alcohol 98.5:1.5, vortex, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 250 μ L MeCN, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 NovaPak C18

Mobile phase: MeCN:MeOH:50 mM phosphate buffer 40:5:55

Detector: UV 220

CHROMATOGRAM

Limit of detection: 100-200 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

von Moltke, L.L.; Greenblatt, D.J.; Harmatz, J.S.; Duan, S.X.; Harrel, L.M.; Cotreau-Bibbo, M.M.; Pritchard, G.A.; Wright, C.E.; Shader, R.I. Triazolam biotransformation by human liver microsomes in vitro: Effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole. *J.Pharmacol.Exp.Ther.*, **1996**, 276, 370-379

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 500 μ L MeCN, mix, centrifuge, inject a 200 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 mm long C18

Column: 250 \times 4.6 5 μ m C18 (Beckman)

Mobile phase: MeCN:50 mM pH 2.2 phosphoric acid 40:60

Flow rate: 2

Injection volume: 200

Detector: UV 207

CHROMATOGRAM

Limit of quantitation: 20 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Chin, T.W.F.; Loeb, M.; Fong, I.W. Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrob.Agents Chemother.*, **1995**, 39, 1671-1675

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 300 μ L 5 μ g/mL terconazole in MeCN, vortex, centrifuge, inject 75 μ L supernatant.

HPLC VARIABLES

Column: 300 \times 4.5 μ Bondapak C18

Mobile phase: MeOH:buffer 60:40 (Buffer was 25 mM KH_2PO_4 + 4 mM heptanesulfonic acid, adjusted to pH 8.0 with 1 M NaOH.)

Flow rate: 1.8

Injection volume: 75

Detector: UV 226

CHROMATOGRAM

Internal standard: terconazole

Limit of quantitation: 10 ng/mL

KEY WORDS

serum

REFERENCE

Carver, P.L.; Berardi, R.R.; Knapp, M.J.; Rider, J.M.; Kauffman, C.A.; Bradley, S.F.; Atassi, M. In vivo interaction of ketoconazole and sucralfate in healthy volunteers. *Antimicrob.Agents Chemother.*, 1994, 38, 326-329

SAMPLE

Matrix: blood

Sample preparation: Condition a C18 SPE cartridge (Analytichem part 607303) by washing with 2 mL MeOH then 5 mL water. 1 mL Serum + 100 μ L 3 mg/mL clotrimazole in MeOH + 200 μ L ammonium hydroxide, add to cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the residue to dryness under a stream of nitrogen at 45 $^\circ$, reconstitute in 1 mL mobile phase, inject 50 μ L aliquot. (SPE preparation from J. Chromatogr. 1986, 377, 287.)

HPLC VARIABLES

Column: 150 \times 3.9 Novopak C18

Mobile phase: MeOH:MeCN:20 mM KH_2PO_4 30:30:35, adjusted to pH 6.8

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 4.3

Internal standard: clotrimazole (7.0)

Limit of quantitation: 200 ng/mL

KEY WORDS

serum; SPE

REFERENCE

Piscitelli, S.C.; Goss, T.F.; Wilton, J.H.; D'Andrea, D.T.; Goldstein, H.; Schentag, J.J. Effects of ranitidine and sucralfate on ketoconazole bioavailability. *Antimicrob.Agents Chemother.*, 1991, 35, 1765-1771

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond-Elut C18 cartridge with 6 mL MeOH and 6 mL water. 1 mL Serum + 110 μ L 50 mg/L terconazole in water + 250 μ L 100 mM NaOH + 3 mL water, add to cartridge, wash with 9 mL water, wash with 200 μ L MeOH, elute

with 1 mL MeOH. Evaporate eluent at 60°, resuspend in 200 μ L mobile phase, centrifuge at 13000 g for 2 min, inject 20-40 μ L.

HPLC VARIABLES

Guard column: Chrompack C18

Column: 100 \times 3 Hypersil ODS in a Chrompack glass cartridge

Mobile phase: MeCN:water 45:55 containing 500 μ L/L diethylamine, pH adjusted to 8.0 with orthophosphoric acid

Flow rate: 0.6

Injection volume: 20-40

Detector: UV 254

CHROMATOGRAM

Retention time: 5.0

Internal standard: terconazole (11.4)

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, acyclovir, allopurinol, amoxicillin, amphotericin B, ampicillin, aspirin, azlocillin, bendrofluazide, bumetanide, buprenorphine, carbenicillin, cefazolin, cefotaxime, cefoxitin, ceftazidime, cefuroxime, cephalixin, chlorambucil, chloramphenicol, chlordiazepoxide, chlorpheniramine, chlorpropamide, cyclophosphamide, cyclosporin, cytarabine, daunorubicin, dextropropoxyphene, dihydrocodeine, domperidone, flucytosine, furosemide, gentamicin, griseofulvin, melphalan, methotrexate, metochlorpramide, metronidazole, miconazole, nabilone, netilmicin, nicotinamide, nitrazepam, penicillin G, piperacillin, prednisolone, procarbazine, prochlorperazine, riboflavin, rifampin, sulfamethoxazole, thioguanine, tobramycin, tolbutamide, trimethoprim

Interfering: diazepam

KEY WORDS

serum

REFERENCE

Turner, C.A.; Turner, A.; Warnock, D.W. High performance liquid chromatographic determination of ketoconazole in human serum. *J.Antimicrob.Chemother.*, **1986**, *18*, 757-763

SAMPLE

Matrix: blood, tissue

Sample preparation: Condition a SPICE reversed-phase SPE cartridge by washing with 2 mL MeOH then 5 mL water. Tissue. 0.5 g Tissue + 100 μ L 3 mg/mL clotrimazole in MeOH + 4 mL MeCN, homogenize for 2 min using a PTFE pestle in a tissue grinder, centrifuge at 1500 g for 15 min. Remove supernatant and evaporate it under a stream of nitrogen at 45°. Reconstitute residue in 1 mL 10 mM HCl, add 200 μ L ammonium hydroxide to adjust pH to about 10.5, add to cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the residue to dryness under a stream of nitrogen at 45°, reconstitute in 200 μ L mobile phase, inject 50 μ L aliquot. Plasma. 1 mL Plasma + 100 μ L 3 mg/mL clotrimazole in MeOH + 200 μ L ammonium hydroxide, add to cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the residue to dryness under a stream of nitrogen at 45°, reconstitute in 1 mL mobile phase, inject 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m Novapak C18

Mobile phase: MeCN:MeOH:20 mM pH 6.8 KH₂PO₄/NaOH 30:35:35

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM**Retention time:** 4.5**Internal standard:** clotrimazole (6:8)**Limit of detection:** 200 ng/mL (plasma), 400 ng/g (tissue)

KEY WORDS

plasma; SPE; lung; liver; adrenal

REFERENCERiley, C.M.; James, M.O. Determination of ketoconazole in the plasma, liver, lung and adrenal of the rat by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *377*, 287–294

SAMPLE**Matrix:** formulations**Sample preparation:** Tablets. Powder tablets, weigh out amount equivalent to about 30 mg ketoconazole, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 200 µg/mL clotrimazole 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 MeOH:buffer 85:15. Add eluate to 1 mL 200 µg/mL clotrimazole in MeOH, make up to 5 mL with MeOH, inject 20 µL aliquot. (Buffer was 50 mM triethylamine adjusted to pH 7.0 with phosphoric acid.)

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 [Enhanced sensitivity with photoreactor (Beam Boost model C6808 with 10 m × 0.3 mm reaction coil) followed by UV detection at 270 nm.]

CHROMATOGRAM**Retention time:** 7**Internal standard:** clotrimazole (9.5)

OTHER SUBSTANCES**Simultaneous:** bifonazole, econazole, fenticonazole, isoconazole, miconazole, tioconazole

KEY WORDS

tablets; creams; post-column reaction

REFERENCEDi 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:** 5 µm Deltabond CN (Keystone)**Mobile phase:** Carbon dioxide:MeOH

Flow rate: 0.5 (CO₂), 0.05 to 0.12 in 7 min (MeOH)

Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

outlet pressure 3600 psi; SFC; back-pressure regulator heated to 60°

REFERENCE

Ashraf-Khorassani, M.; Levy, J.M. Addition of modifier in supercritical fluid chromatography using a microbore reciprocating pump. *Chromatographia*, **1995**, *40*, 78–84

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: 11.30 (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, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, pimizide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protripty-

line, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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: Skin sample extracted with 250 μ L mobile phase, vortex 1 min, centrifuge at 8000 rpm for 10 min, inject 40 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.5 Whatman 5 μ m reverse-phase C18

Mobile phase: MeCN:10 mM pH 6.0 K_2HPO_4 65:35

Flow rate: 0.7

Injection volume: 40

Detector: UV 254

CHROMATOGRAM

Retention time: 8.6

Limit of detection: 50 ng/mL

KEY WORDS

skin

REFERENCE

Pershing, L.K.; Corlett, J.; Jorgensen, C. In vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum corneum. *Antimicrob.Agents Chemother.*, **1994**, 38, 90–95

ANNOTATED BIBLIOGRAPHY

Hoffman, D.W.; Jones-King, K.L.; Ravaris, C.L.; Edkins, R.D. Electrochemical detection for high-performance liquid chromatography of ketoconazole in plasma and saliva. *Anal.Biochem.*, **1988**, 172, 495–498

Badcock, N.R. Micro-determination of ketoconazole in plasma or serum by high-performance liquid chromatography. *J.Chromatogr.*, **1984**, 306, 436–440

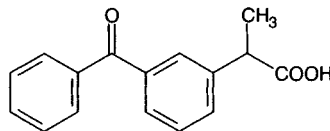
Pascucci, V.L.; Bennett, J.; Narang, P.K.; Chatterji, D.C. Quantitation of ketoconazole in biological fluids using high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, 72, 1467–1469

Ketoprofen

Molecular formula: C₁₆H₁₄O₃

Molecular weight: 254.3

CAS Registry No.: 22071-15-4



SAMPLE

Matrix: bile, blood, perfusate

Sample preparation: Dilute bile with saline. Inject 200 μ L plasma, 100 μ L perfusate, or 100 μ L diluted bile onto column A and elute to waste with mobile phase A, after 15 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 and monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 5 min before the next injection. (See also *J.Pharm.Sci.* 1995, 84, 1327.)

HPLC VARIABLES

Column: A 30 \times 4.6 L-column (porous silica gel with internal surfaces coated with octadecyl groups and external surfaces coated with glycerylpropyl groups) (Chemical Inspection and Testing Institute, Tokyo); B 250 \times 4.6 Sumichiral OA-2500S ((R)-N-(3,5-dinitrobenzoyl)-1-naphthylglycine bonded to aminopropyl silica) (Sumika, Osaka)

Mobile phase: A 20 mM pH 6.8 ammonium acetate buffer; B MeOH:buffer 95:5, pH 6.2 (Buffer was 1 M acetic acid:1 M ammonium acetate 20:80, pH 4.0. Dilute to 600 mM acetate before use.)

Flow rate: 1

Injection volume: 100-200

Detector: UV 262

CHROMATOGRAM

Retention time: 27 (-), 29 (+)

Limit of detection: 20 ng/mL

KEY WORDS

chiral; rat; column-switching; plasma

REFERENCE

Yagi, M.; Shibukawa, A.; Nakagawa, T. Direct injection analysis of ketoprofen enantiomers in plasma using column-switching high-performance liquid chromatography system. *Chem.Pharm.Bull.*, **1990**, 38, 2513-2517

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L tolmetin solution + 500 μ L pH 1.8 phosphate buffer, extract with 1-butanol/MTBE. Remove the organic layer and add it to 500 μ L pH 6.1 ammonium acetate buffer, mix, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Cosmosil C18

Mobile phase: MeCN:250 mM pH 5.0 ammonium acetate buffer 20:80

Flow rate: 1.8

Detector: UV 350

CHROMATOGRAM

Internal standard: tolmetin (UV 258)

Limit of quantitation: 5 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Shah, A.K.; Wei, G.; Lanman, R.C.; Bhargava, V.O.; Weir, S.J. Percutaneous absorption of ketoprofen from different anatomical sites in man. *Pharm.Res.*, **1996**, *13*, 168–172

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 500 μ L MeCN, vortex for 10 s, centrifuge at 1500 g for 10 min. Remove 800 μ L of the supernatant and add it to 5 mL dichloromethane, vortex for 30 s, centrifuge for 10 min, inject a 50 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 20 \times 4.6 10 μ m Spherisorb C8

Column: 250 \times 4.6 5 μ m Spherisorb ODS

Mobile phase: MeOH:buffer 40:60 (Buffer was 40 mM Na₂HPO₄ adjusted to pH 8 with orthophosphoric acid.)

Column temperature: 50

Flow rate: 1

Injection volume: 50

Detector: UV 360

CHROMATOGRAM

Retention time: 8.6

Internal standard: ketoprofen

OTHER SUBSTANCES

Extracted: piroxicam

KEY WORDS

plasma; ketoprofen is IS

REFERENCE

Edno, L.; Bressolle, F.; Combe, B.; Galtier, M. A reproducible and rapid HPLC assay for quantitation of piroxicam in plasma. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 785–789

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 125 μ L 40 mM decanoic acid in MeCN, mix. Dialyze a 100 μ L sample against 20 mM pH 7.0 phosphate buffer using a Gilson Cuprophane membrane (molecular mass cut-off 15 kDa). Continuously pump the buffer through the dialysis cell and through column A at 3 mL/min for 9.6 min, backflush the contents of column A onto column B with the mobile phase, monitor the effluent from column B. (After each injection flush plasma channel with 1 mL 0.05% Triton X-100, with 1 mL 1 mM HCl, and with 2 mL water. After each injection flush buffer channel with 3 mL 20 mM pH 7.0 phosphate buffer and condition column A with 1 mL 20 mM pH 7.0 phosphate buffer.)

HPLC VARIABLES

Column: A 10 \times 2 40 μ m Bondesil C18 (Analytichem); B 250 \times 3.1 5 μ m C18 (RoSil Research Separation Laboratories)

Mobile phase: MeCN:MeOH:20 mM pH 3.2 phosphate buffer 50:10:40

Flow rate: 1

Injection volume: 100

Detector: UV 261

CHROMATOGRAM

Retention time: 5.5

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: fenoprofen (UV 272), flurbiprofen (UV 247), ibuprofen (UV 264), naproxen (UV 272)

KEY WORDS

plasma; dialysis; column-switching

REFERENCE

Herráez-Hernández, R.; Van de Merbel, N.C.; Brinkman, U.A.T. Determination of the total concentration of highly protein-bound drugs in plasma by on-line dialysis and column liquid chromatography: application to non-steroidal anti-inflammatory drugs. *J.Chromatogr.B*, **1995**, 666, 127–137

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 100 μ L 50 μ g/mL indomethacin + 500 μ L 600 mM sulfuric acid + 15 mL dichloromethane, mix for 20 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μ L 50 mM triethylamine in MeCN + 50 μ L 60 mM ethyl chloroformate in MeCN, vortex for 30 s, add 50 μ L 100 mM L-leucinamide in MeOH:triethylamine 100:14, let stand for 2 min, add 50 μ L water, inject a 10-50 μ L aliquot of the reaction mixture.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrabase C18 (Shandon)

Mobile phase: MeCN:60 mM KH_2PO_4 :triethylamine 49:51:0.1

Flow rate: 1.8

Injection volume: 10-50

Detector: UV 275

CHROMATOGRAM

Retention time: 2.0 (R-(-)), 2.5 (S-(+))

Internal standard: indomethacin (5.3)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: flurbiprofen, ibuprofen (UV 225)

KEY WORDS

plasma; chiral; derivatization

REFERENCE

Péhourecq, F.; Lagrange, F.; Labat, L.; Bannwarth, B. Simultaneous measurement of flurbiprofen, ibuprofen, and ketoprofen enantiomer concentrations in plasma using L-leucinamide as the chiral coupling component. *J.Liq.Chromatogr.*, **1995**, 18, 3969–3979

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)) KH₂PO₄ 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 257

CHROMATOGRAM

Retention time: 4.69

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amoxapine, aspirin, astemizole, atenolol, benazepril, benzocaine, benzoyllecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, dembrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mephenesin, mephentermine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfinyprazole, sulindac, sultopride, sultopride, suriclone, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, vinblastine, vincristine, videsine, warfarin, yohimbine, zopiclone, zorubicine

Interfering: alminoprofen, amodiaquine, benperidol, chloroquine, cicletanine, cocaine, doxylamine, droperidol, hydroxychloroquine, ketoprofen, labetalol, meperidine, mepivacaine, moclobemide, nomifensine, temazepam, timolol, viloxazine, zolpidem

KEY WORDSwhole blood; plasma

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 Plasma + 100 μL 20 $\mu\text{g}/\text{mL}$ ibuprofen + 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 \times 6 Shimpack CLS-ODS (Shimadzu)**Mobile phase:** MeCN:MeOH:0.5 mM phosphoric acid 30:30:40**Column temperature:** 40**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 258

CHROMATOGRAM**Internal standard:** ibuprofen

KEY WORDSplasma; 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:** 1 mL Plasma + 50 μL 200 $\mu\text{g}/\text{mL}$ S-naproxen in MeOH + 500 μL 2 M sulfuric acid + 8 mL n-hexane:ethyl acetate 90:10, mix gently at 30 rpm for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and evaporate it to dryness at 45° under a stream of nitrogen. Reconstitute in 100 μL 1.5% thionyl chloride in n-hexane (freshly prepared), heat at 75° for 1 h in a capped tube, cool to room temperature, add 500 μL 2% S-1-phenylethylamine in dichloromethane (freshly prepared), let stand for 15 min, add 500 μL 2 M sulfuric acid + 5 mL n-hexane, mix gently at 30 rpm for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and evaporate it to dryness at 45° under a stream of nitrogen. Reconstitute in 250 μL mobile phase, inject 200 μL aliquot.

HPLC VARIABLES**Column:** 250 \times 4 5 μm SGE silica glass column**Mobile phase:** n-Heptane:isopropanol 92:8**Flow rate:** 1**Injection volume:** 200**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5.2(R), 6.6(S)**Internal standard:** S-naproxen (5.9)**Limit of quantitation:** 150 ng/mL

OTHER SUBSTANCES

Simultaneous: fenoprofen, ibuprofen, mefenamic acid, salicylic acid

Noninterfering: diazepam, digoxin, methylprednisolone, midazolam, nifedipine, penicillamine, ranitidine, theophylline

KEY WORDS

plasma; normal phase; derivatization; chiral

REFERENCE

Hayball, P.J.; Nation, R.L.; Bochner, F.; Le Leu, R.K. Enantiospecific analysis of ketoprofen in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, 570, 446–452

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 200 μ L 2 M HCl + 6 mL ice-cold diethyl ether, extract, centrifuge at 1500 g for 10 min. Remove 5 mL of organic layer and evaporate it to dryness under a stream of nitrogen. Dissolve in 250 μ L isopropanol:water 2:8, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.0 AGP (EnantioPac)

Mobile phase: 20 mM pH 6.7 phosphate buffer containing 0.5% isopropanol and 5 mM dimethyloctylamine

Column temperature: 15

Flow rate: 0.5

Injection volume: 40

Detector: UV 260

CHROMATOGRAM

Retention time: 25(R), 32(S)

Limit of quantitation: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Menzel-Soglowek, S.; Geisslinger, G.; Brune, K. Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1 -acid glycoprotein column. *J.Chromatogr.*, **1990**, 532, 295–303

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg C2 SPE cartridge (Analytichem) with 2 mL MeOH and 1 mL water. 100 μ L Plasma + 20 μ L MeOH + 500 μ L 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 μ L aliquot.

HPLC VARIABLES

Guard column: 30-40 μ m pellicular Vydac Reversed-Phase

Column: 75 \times 3.9 4 μ m Nova-Pak phenyl

Mobile phase: MeOH:buffer 42:58 (Buffer was 100 mM NaH₂PO₄ 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: 2.7

Internal standard: ketoprofen

Limit of detection: 23 ng/mL

OTHER SUBSTANCES

Extracted: clofibrac acid

KEY WORDS

post-column reaction; plasma; SPE; ketoprofen is IS

REFERENCE

Bachman, W.J.; Stewart, J.T. HPLC-photolysis-electrochemical detection in pharmaceutical analysis: Application to the determination of clofibrac acid in human plasma. *J.Liq.Chromatogr.*, **1989**, *12*, 2947-2959

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 500 µL 4-8 µg/mL IS in water + 500 µL buffer + 4 mL dichloromethane:n-propanol 99:1, extract on a rotamixer, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, add 5 drops toluene, evaporate to dryness under a stream of air at 30°, reconstitute the residue in 200 µL 50 mM triethylamine in MeCN, add 100 µL 60 mM ethyl chloroformate in MeCN, after 30 s add 100 µL 1 M l-leucinamide hydrochloride in MeOH containing 1 M triethylamine, after 2 min add 500 µL 250 mM HCl, extract with 4 mL ethyl acetate. Evaporate the organic layer to dryness under a stream of air at 30°, reconstitute the residue with 100 µL MeCN, add 400 µL 10 mM pH 6.5 phosphate buffer, inject a 60 µL aliquot. (Prepare buffer as follows. Neutralize a 1 M solution of tetrabutylammonium sulfate in water with NaOH, wash 5 times with dichloromethane, wash twice with heptane. Prepare a 100 mM pH 9.6 sodium carbonate buffer containing 0.5 M of the neutralized and washed tetrabutylammonium salt.)

HPLC VARIABLES

Guard column: 30 × 4 Perisorb RP-18 (Merck)

Column: 250 × 4 7 µm LiChroCart RP-18 (Merck)

Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 38:62

Flow rate: 2

Injection volume: 10

Detector: UV 260

CHROMATOGRAM

Retention time: 8.5 (-), 10 (+)

Internal standard: 2-(4-benzoylphenyl)butyric acid (12, 15 (enantiomers))

Limit of quantitation: 250 ng/mL

KEY WORDS

plasma; derivatization; chiral; pharmacokinetics

REFERENCE

Björkman, S. Determination of the enantiomers of ketoprofen in blood plasma by ion-pair extraction and high-performance liquid chromatography of leucinamide derivatives. *J.Chromatogr.*, **1987**, *414*, 465-471

SAMPLE**Matrix:** blood**Sample preparation:** Condition a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply to cartridge. Wash with 100 μ L water, elute with three 500 μ L portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.5 5 μ m Spherisorb ODS**Mobile phase:** MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid.)**Flow rate:** 1**Injection volume:** 20**Detector:** UV 250

CHROMATOGRAM**Retention time:** 5

OTHER SUBSTANCES**Simultaneous:** acetaminophen, fenoprofen, ibuprofen, indomethacin, naproxen, salicylic acid

KEY WORDS

whole blood; SPE

REFERENCEMoore, C.M.; Tebbett, I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis. *Forensic Sci.Int.*, **1987**, *34*, 155-158

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 200 μ L 1 M HCl + 4-5 mL ethyl acetate, vortex for 1.5-2 min, centrifuge at 400 g for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 40-50°, reconstitute the residue in 200 μ L MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 100 \times 5 10 μ m spherical C18 radial compression (Waters)**Mobile phase:** MeCN:water 45:55 containing 2.5 mL/L acetic acid**Flow rate:** 1**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 5**Internal standard:** ketoprofen

OTHER SUBSTANCES**Extracted:** oxaprozin**Simultaneous:** acetaminophen, fenoprofen, flurbiprofen, indomethacin, phenylbutazone, salicylic acid**Noninterfering:** ibuprofen, piroxicam

KEY WORDS

plasma; ketoprofen is IS

REFERENCE

Matlis, R.; Greenblatt, D.J. Rapid high-performance liquid chromatographic analysis of oxaprozin, a non-steroidal anti-inflammatory agent. *J.Chromatogr.*, **1984**, *310*, 445–449

SAMPLE**Matrix:** blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 10 μ g fenoprofen + 100 μ L 600 mM sulfuric acid + 4 mL 2,2,4-trimethylpentane:isopropanol 95:5, vortex for 10 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 180 μ L mobile phase, vortex for 10 s, inject a 100 μ L aliquot. Urine. 500 μ L Urine + 20 μ g fenoprofen + 100 μ L 600 mM sulfuric acid + 4 mL 2,2,4-trimethylpentane:isopropanol 95:5, vortex for 10 s, centrifuge at 1800 g for 3 min. Remove the organic layer and add it to 3 mL water, vortex for 10 s, centrifuge for 3 min. Remove the aqueous phase and add it to 200 μ L 600 mM sulfuric acid and 3 mL chloroform, vortex for 10 s, centrifuge for 3 min. Remove the organic phase and evaporate it to dryness, reconstitute the residue in 180 μ L mobile phase, vortex for 10 s, inject a 100 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Chiralpak AD amylose carbamate (Chiral Technologies)**Mobile phase:** Hexane:isopropanol:trifluoroacetic acid 80:19.9:0.1**Flow rate:** 1**Injection volume:** 100**Detector:** UV 254**CHROMATOGRAM****Retention time:** 7.0 (R(-)), 8.3 (S(+))**Internal standard:** fenoprofen (5.3, 6.3 (enantiomers))**Limit of quantitation:** 50 ng/mL (plasma); 200 ng/mL (urine)**KEY WORDS**

plasma; chiral

REFERENCE

Carr, R.A.; Cail , G.; Ngoc, A.H.; Foster, R.T. Stereospecific high-performance liquid chromatographic assay of ketoprofen in human plasma and urine. *J.Chromatogr.B*, **1995**, *668*, 175–181

SAMPLE**Matrix:** blood, urine

Sample preparation: Add naproxen to plasma or urine, acidify with 1 M pH 2 phosphate buffer, extract with hexane:THF 80:20.

HPLC VARIABLES**Guard column:** 20 \times 4.6 Nucleosil OCS 10**Column:** 150 \times 6 YMC Pack A312 S5 120A ODS**Mobile phase:** MeCN:50 mM phosphate buffer 19:83**Column temperature:** 28**Flow rate:** 2**Injection volume:** 50**Detector:** UV 262**CHROMATOGRAM****Retention time:** 14.48 (plasma), 13.53 (urine)

Internal standard: naproxen (11.11 (plasma), 10.47 (urine))

Limit of quantitation: 20 ng/mL

KEY WORDS

plasma; rat

REFERENCE

Daffonchio, L.; Bestetti, A.; Clavenna, G.; Fedele, G.; Ferrari, M.P.; Omini, C. Effect of a new foam formulation of ketoprofen lysine salt in experimental models of inflammation and hyperalgesia. *Arzneimittelforschung*, **1995**, *45*, 590–594

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μ L Plasma + 100 μ L 600 mM sulfuric acid + 4 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and add 4 mL water to it. Vortex for 30 s, centrifuge for 3 min. Remove organic layer and evaporate it to dryness on a Speed Vac concentrator. Reconstitute residue in 200 μ L MeOH, vortex 30 s, add 100 μ L 100 μ g/mL indoprofen in water, inject a 20 μ L aliquot. Urine. 100 μ L Urine + 25 μ L 1 M NaOH, add 125 μ L 600 mM sulfuric acid, proceed as for plasma.

HPLC VARIABLES

Guard column: 50 \times 5 37-53 μ m C18 material

Column: 100 \times 4.6 5 μ m Partisil 5 ODS-3

Mobile phase: MeCN:60 mM KH_2PO_4 :triethylamine 25:75:0.1

Flow rate: 1

Injection volume: 20

Detector: UV 275

CHROMATOGRAM

Retention time: 4.7

Internal standard: indoprofen (3.4)

OTHER SUBSTANCES

Simultaneous: probenecid

KEY WORDS

plasma; rat

REFERENCE

Palylyk, E.L.; Jamali, F. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, *568*, 187–196

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μ L Plasma + 100 μ L 100 μ g/mL indoprofen in water + 100 μ L 600 mM sulfuric acid + 5 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and add 5 mL water to it. Vortex for 30 s, centrifuge for 3 min. Remove organic layer and evaporate it to dryness on a Speed Vac concentrator. Reconstitute residue in 100 μ L 50 mM triethylamine in MeCN, vortex 30 s, add 50 μ L 60 mM ethyl chloroformate in MeCN, let stand 30 s, add 50 μ L 1 M L-leucinamide hydrochloride and 1 M triethylamine in MeOH, let stand 2 min, add 50 μ L water, inject 10-60 μ L aliquots. Urine. 100 μ L Urine + 25 μ L 1 M NaOH, add 125 μ L 600 mM sulfuric acid, proceed as for plasma.

HPLC VARIABLES

Guard column: 50 × 5 37-53 μm C18 material

Column: 100 × 4.6 5 μm Partisil 5 ODS-3

Mobile phase: MeCN:60 mM KH₂PO₄:triethylamine 35:65:0.1

Flow rate: 1

Injection volume: 10-60

Detector: UV 275

CHROMATOGRAM

Retention time: 10 (R), 12 (S)

Internal standard: indoprofen (6(R), 7(S))

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: probenecid

Also analyzed: carprofen, cicloprofen, fenoprofen, flurbiprofen, indoprofen, pirprofen

KEY WORDS

plasma; rat; chiral; derivatization

REFERENCE

Palylyk, E.L.; Jamali, F. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, 568, 187–196

SAMPLE

Matrix: blood, urine

Sample preparation: 50 μL Plasma or urine + 10 μL 1 M NaOH, heat at 37° for 2 h, add 10 μL 1 M HCl, extract with 1 mL ethyl acetate. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 50 mM triethylamine in MeCN, add 50 μL 60 mM ethyl chloroformate in MeCN, let stand for 2 min, add 50 μL 1 M L-leucinamide in 1 M triethylamine in MeOH. Evaporate, take up the residue in 100 μL mobile phase, inject a 100 μL aliquot. (Hydrolysis of glucuronides may be omitted.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS

Mobile phase: MeCN:60 mM pH 6 potassium phosphate buffer 40:60

Flow rate: 1

Injection volume: 100

Detector: UV 272

CHROMATOGRAM

Retention time: 13.6 (R), 16.0 (S)

Internal standard: ketoprofen

OTHER SUBSTANCES

Extracted: fenoprofen, flunoxaprofen

KEY WORDS

plasma; chiral; ketoprofen is IS

REFERENCE

Volland, C.; Sun, H.; Benet, L.Z. Stereoselective analysis of fenoprofen and its metabolites. *J.Chromatogr.*, **1990**, 534, 127–138

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μL Plasma + 100 μL 100 $\mu\text{g}/\text{mL}$ calcium fenoprofen in water + 100 μL 600 mM sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 1800 RCF for 5 min. Remove the organic layer and add it to 3 mL water, vortex for 30 s, centrifuge for 3 min. Remove the aqueous layer and add it to 200 μL 600 mM sulfuric acid, add 3 mL chloroform, vortex for 30 s, centrifuge for 3 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute with 100 μL 50 mM triethylamine in MeCN, add 50 μL 60 mM ethyl chloroformate in MeCN, after 30 s add 50 μL 1 M l-leucinamide hydrochloride in MeOH containing 1 M triethylamine, after 2 min add 50 μL water, inject a 10-40 μL aliquot. Urine. 100-500 μL Urine + 25-125 μL 1 M NaOH, mix, add a volume of 600 mM sulfuric acid equal to the volume of 1 M NaOH plus 100 μL , add 100 μL 100 $\mu\text{g}/\text{mL}$ calcium fenoprofen in water, add 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 1800 RCF for 5 min. Remove the organic layer and add it to 3 mL water, vortex for 30 s, centrifuge for 3 min. Remove the aqueous layer and add it to 200 μL 600 mM sulfuric acid, add 3 mL chloroform, vortex for 30 s, centrifuge for 3 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute with 100 μL 50 mM triethylamine in MeCN, add 50 μL 60 mM ethyl chloroformate in MeCN, after 30 s add 50 μL 1 M l-leucinamide hydrochloride in MeOH containing 1 M triethylamine, after 2 min add 50 μL water, inject a 10-40 μL aliquot.

HPLC VARIABLES

Guard column: 50 mm long 37-53 μm C18

Column: 100 mm long Partisil 5 ODS-3

Mobile phase: MeCN:60 mM KH_2PO_4 :triethylamine 64:36:0.02

Flow rate: 1

Injection volume: 10-40

Detector: UV 275

CHROMATOGRAM

Retention time: 9.8 (R(-)), 11.3 (S(+))

Internal standard: fenoprofen 17.7 (R(-)), 19.9 (S(+))

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: flurbiprofen

Interfering: naproxen

KEY WORDS

plasma; derivatization; chiral

REFERENCE

Foster, R.T.; Jamali, F. High-performance liquid chromatographic assay of ketoprofen enantiomers in human plasma and urine. *J.Chromatogr.*, **1987**, *416*, 388-393

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 350 μL 2 $\mu\text{g}/\text{mL}$ Naproxen in 10 mM pH 6.0 phosphate buffer containing 0.05% MeOH + 650 μL pH 6 phosphate buffer + 1 mL plasma + 0.5 mL 1 M pH 2 phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μL mobile phase, vortex for 15 s, inject aliquot. Urine. 350 μL 20 $\mu\text{g}/\text{mL}$ Naproxen in 10 mM pH 6.0 phosphate buffer containing 0.5% MeOH + 650 μL pH 6 phosphate buffer + 1 mL urine + 1 mL 0.5 M pH 7 phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μL mobile phase, vortex for 15 s, inject aliquot.

HPLC VARIABLES

Guard column: 40 × 3.2 30-44 μm Vydac reverse-phase
Column: 40 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 6:94 to 8:92
Flow rate: 2
Injection volume: 5-200
Detector: UV 262

CHROMATOGRAM

Retention time: 16
Internal standard: naproxen (10)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, fenoprofen, probenecid, salicylic acid

KEY WORDS

plasma

REFERENCE

Upton, R.A.; Buskin, J.N.; Guentert, T.W.; Williams, R.L.; Riegelman, S. Convenient and sensitive high-performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine. *J.Chromatogr.*, **1980**, *190*, 119-128

SAMPLE

Matrix: bulk

Sample preparation: 10 mg Compound + 10 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride + 2 drops 3,5-dimethylaniline + 1.5 mL dichloromethane, mix, after 30 min add 1 mL 1 M HCl, shake vigorously. Remove the lower organic layer and dry it over anhydrous magnesium sulfate, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm D N-(3,5-dinitrobenzoyl)phenylglycine (Regis)
Mobile phase: Hexane:isopropanol 80:20
Flow rate: 2
Injection volume: 20
Detector: UV 254; UV 280

CHROMATOGRAM

Retention time: k' 4.97 (for first enantiomer)

OTHER SUBSTANCES

Also analyzed: carprofen, cicloprofen, etodolac, fenoprofen, flurbiprofen, ibuprofen, naproxen, piroprofen, tiaprofenic acid

KEY WORDS

derivatization; $\alpha = 1.18$; chiral

REFERENCE

Pirkle, W.H.; Murray, P.G. The separation of the enantiomers of a variety of non-steroidal anti-inflammatory drugs (NSAIDs) as their anilide derivatives using a chiral stationary phase. *J.Liq.Chromatogr.*, **1990**, *13*, 2123-2134

SAMPLE

Matrix: formulations

Sample preparation: Weigh out capsule contents equivalent to 50 mg ketoprofen, add 80 mL MeOH, sonicate for 10 min, make up to 100 mL with MeOH, filter. Dilute a 4 mL aliquot of the filtrate to 100 mL with mobile phase. Mix a 10 mL aliquot of the diluted solution with 10 mL 50 $\mu\text{g/mL}$ ibuprofen in mobile phase, make up to 100 mL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μm Spheri-5 RP-8

Mobile phase: MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na_2HPO_4 and 7 mM KH_2PO_4 to achieve pH 7.)

Flow rate: 1

Injection volume: 50

Detector: F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 $\mu\text{g/mL}$ reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m \times 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α -(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetoneitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α -(3,4-dimethoxyphenyl)-4'-methylcinnamitrile. Dissolve 20 mmoles α -(3,4-dimethoxyphenyl)-4'-methylcinnamitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h, cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α -(3,4-dimethoxyphenyl)-4'-bromomethylcinnamitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α -(3,4-dimethoxyphenyl)-4'-bromomethylcinnamitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α -(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α -(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α -(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM

Retention time: k' 1.5504

Internal standard: ibuprofen (k' 4.124)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: mefenamic acid, naproxen, probenecid, salicylic acid, valproic acid

KEY WORDS

capsules; post-column extraction

REFERENCE

Kim, M.; Stewart, J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α -phenylcinnamitrile quaternary ammonium salt as a new fluorescent ion-pair reagent. *J.Liq.Chromatogr.*, **1990**, *13*, 213-237

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 3 Ecocart LiChrospher 100 RP-18

Mobile phase: Isopropanol:100 mM KH₂PO₄:formic acid 54:100:0.1

Flow rate: 0.6

Detector: UV 254

CHROMATOGRAM

Retention time: 4.3

Limit of quantitation: 200-500 ng/mL

OTHER SUBSTANCES

Also analyzed: acemetacin, diclofenac, flurbiprofen, indomethacin, lonazolac, 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: 250 × 4.5 5 μm Ultrasphere ODS

Mobile phase: MeCN:10 mM tetrabutylammonium buffer 45:55

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 11.6

OTHER SUBSTANCES

Simultaneous: fenoprofen

REFERENCE

Bischer, A.; Iwaki, M.; Zia-Amirhosseini, P.; Benet, L.Z. Stereoselective reversible binding properties of the glucuronide conjugates of fenoprofen enantiomers to human serum albumin. *Drug Metab.Dispos.*, **1995**, *23*, 900-903

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: 7.02 (A), 7.04 (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, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, pimozone, 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, sulfapyrazone, 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

some 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 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 \times 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 \times 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 \times 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)

Mobile phase: 50 mM pH 5.5 KH_2PO_4

Flow rate: 0.8

Injection volume: 20

Detector: UV

CHROMATOGRAM

Retention time: k' 2.56

OTHER SUBSTANCES

Simultaneous: flurbiprofen, isradipine, nimodipine, suprofen

KEY WORDS

chiral; $\alpha = 1.22$

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

SAMPLE

Matrix: solutions

Sample preparation: 1 mL 1.23 mg/mL ketoprofen in dichloromethane + 300 μL 1 mg/mL hydroxybenzotriazole in dichloromethane:pyridine 99:1 + 300 μL 11 mg/mL 1-ethyl-3-dimethylaminopropylcarbodiimide in dichloromethane + 300 μL 3.47 mg/mL 1-naphthylamine (Caution! 1-Naphthylamine in a carcinogen!) in dichloromethane, vortex, let stand for 1 h, evaporate to dryness under a stream of nitrogen, reconstitute with 5 mL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 2.1 Tolycellulose EXP B101 (tris(4-methylbenzoate)cellulose covalently bonded to 10 μm aminopropylsilica)

Mobile phase: MeOH:buffer 85:15 (Buffer was 14.05 g/L sodium perchlorate adjusted to pH 2.0.)

Flow rate: 0.21

Injection volume: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 5.9, 8.6 (enantiomers)

Limit of detection: 100 pg

OTHER SUBSTANCES

Also analyzed: fenoprofen, flurbiprofen, ibuprofen, tiaprofenic acid

KEY WORDS

derivatization; narrow-bore; chiral

REFERENCE

Van Overbeke, A.; Baeyens, W.; Van Der Weken, G.; Van de Voorde, I.; Dewaele, C. Comparative chromatographic study on the chiral separation of the 1-naphthylamine derivative of ketoprofen on cellulose-based columns of different sizes. *Biomed.Chromatogr.*, **1995**, 9, 289–290

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 500 $\mu\text{g}/\text{mL}$ solution in isopropanol, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 Chiralcel OJ**Mobile phase:** n-Hexane:isopropanol:acetic acid 80:20:0.5**Flow rate:** 1**Injection volume:** 20**Detector:** UV 230

CHROMATOGRAM**Retention time:** 8, 10 (enantiomers)

OTHER SUBSTANCES**Simultaneous:** benoxaprofen, carprofen, ibuprofen, piroprofen, protizinic acid

KEY WORDSchiral

REFERENCE

Van Overbeke, A.; Baeyens, W.; Dewaele, C. Comparative study on the enantiomeric separation of several non-steroidal anti-inflammatory drugs on two cellulose-based chiral stationary phases. *J.Liq.Chromatogr.*, **1995**, *18*, 2427–2443

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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, kynurenic acid, levorphanol, lidocaine, loraze-

pam, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscaphine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 Chiralpak AD (Daicel)

Mobile phase: Carbon dioxide:MeOH 96:4

Column temperature: 30

Flow rate: 2.5

Detector: UV 210

CHROMATOGRAM

Retention time: 13.5, 15 (enantiomers)

OTHER SUBSTANCES

Simultaneous: fenoprofen, ibuprofen, flurbiprofen, naproxen

KEY WORDS

SFC; 250 bar; chiral

REFERENCE

Kot, A.; Sandra, P.; Venema, A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs. *J.Chromatogr.Sci.*, **1994**, *32*, 439-448

SAMPLE

Matrix: solutions

Sample preparation: Mix 1 mL 100 $\mu\text{g/mL}$ compound in dichloromethane with 300 μL 100 $\mu\text{g/mL}$ 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, 300 μL 1.1 mg/mL 1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride in dichloromethane, and 300 μL 300 $\mu\text{g/mL}$ benzylamine in dichloromethane, vortex, let stand at room temperature for 1.5 h, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 10 μm EXP B101 tris(4-methylbenzoate) cellulose on silica (Bio-Rad)
Mobile phase: MeOH:buffer 70:30 (Prepare buffer solution by dissolving 14.05 g sodium perchlorate in water, adjust pH to 2.0, make up to 1 L with water.)
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Retention time: k' 4.06, k' 5.69 (enantiomers)

OTHER SUBSTANCES

Also analyzed: benoxaprofen (MeOH:buffer 80:20), carprofen, fenoprofen, flurbiprofen, ibuprofen, piroprofen, tiaprofenic acid

KEY WORDS

derivatization; chiral

REFERENCE

Van Overbeke, A.; Baeyens, W.; Van den Bossche, W.; Dewaele, C. Separation of 2-arylpropionic acids on a cellulose based chiral stationary phase by RP-HPLC. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 901–909

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Haginaka, J.; Kanasugi, N. Enantioselectivity of bovine serum albumin-bonded columns produced with isolated protein fragments. *J.Chromatogr.A*, **1995**, *694*, 71–80 [chiral; also benzoin, clorazepate, fenoprofen, flurbiprofen, ibuprofen, lorazepam, lormetazepam, oxazepam, pranoprofen, temazepam, warfarin]

Hyun, M.H.; Ryoo, J.-J.; Cho, Y.J.; Jin, J.S. Unusual examples of the liquid chromatographic resolution of racemates. Resolution of π -donor analytes on a π -donor chiral stationary phase. *J.Chromatogr.A*, **1995**, *692*, 91–96 [chiral; also alminoprofen, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, naproxen]

Van Overbeke, A.; Baeyens, W.; Van den Bossche, W.; Dewaele, C. Enantiomeric separation of amide derivatives of some 2-arylpropionic acids by HPLC on a cellulose-based chiral stationary phase. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 911–916 [chiral; derivatization; also flurbiprofen, ibuprofen, tiaprofenic acid]

Haginaka, J.; Murashima, T.; Fujima, H.; Wada, H. Direct injection assay of drug enantiomers in serum on ovomucoid-bonded silica materials by liquid chromatography. *J.Chromatogr.*, **1993**, *620*, 199–204 [direct injection; serum; chiral; also benzoin, chlorpheniramine, oxazepam]

Szász, G.; Budvári-Bárány, Z.; Löre, A.; Radeczky, G.; Shalaby, A. HPLC of antiphlogistic acids on silica dynamically modified with cetylpyridinium chloride. *J.Liq.Chromatogr.*, **1993**, *16*, 2335–2345 [also diclofenac, ibuprofen, ketoprofen, naproxen, nicotinic acid, niflumic acid, salicylic acid]

Benoit, E.; Jaussaud, P.; Besse, S.; Videmann, B.; Courtot, D.; Delatour, P.; Bonnaire, Y. Identification of a benzhydrolic metabolite of ketoprofen in horses by gas chromatography-mass spectrometry and high-performance liquid chromatography. *J.Chromatogr.*, **1992**, *583*, 167–173 [plasma; SPE; gradient; extracted metabolites; LOQ 500 ng/mL; LOD 100 ng/mL; pharmacokinetics; horse]

Mannucci, C.; Bertini, J.; Cocchini, A.; Perico, A.; Salvagnini, F.; Triolo, A. High performance liquid chromatography simultaneous quantitation of ketoprofen and parabens in a commercial gel formu-

- lation. *J.Liq.Chromatogr.*, **1992**, *15*, 327–335 [gels; formulations; column temp 50; simultaneous butyl paraben, ethyl paraben, methyl paraben, propyl paraben]
- Oda, Y.; Asakawa, N.; Yoshida, Y.; Sato, T. On-line determination and resolution of the enantiomers of ketoprofen in plasma using coupled achiral-chiral high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1992**, *10*, 81–87
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- Shibukawa, A.; Terakita, A.; He, J.Y.; Nakagawa, T. High-performance frontal analysis-high-performance liquid chromatographic system for stereoselective determination of unbound ketoprofen enantiomers in plasma after direct sample injection. *J.Pharm.Sci.*, **1992**, *81*, 710–715 [plasma; column-switching; column temp 15; chiral; LOD 1 nM; human; rat]
- Wong, C.-Y.; Yeh, M.-K.; Wang, D.-P. High-performance liquid chromatographic determination of ketoprofen in pharmaceutical dosage forms and plasma. *J.Liq.Chromatogr.*, **1992**, *15*, 1215–1225 [plasma; formulations; isopropylphenazone (IS); rabbit; human; pharmacokinetics]
- Corvetta, A.; Della Bitta, R.; Luchetti, M.M.; Pomponio, G.; Ciuffoletti, V. Tenoxicam and ketoprofen level monitoring with high performance liquid chromatography in patients affected by rheumatoid arthritis. *Clin.Exp.Rheumatol.*, **1991**, *9*, 143–148
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- Wanwimolruk, S.; Wanwimolruk, S.Z.; Zoest, A.R. Sensitive HPLC assay for ketoprofen in human plasma and its application to pharmacokinetic study. *J.Liq.Chromatogr.*, **1991**, *14*, 3685–3694 [plasma; pharmacokinetics; piroxicam (IS); microbore; LOD 50 ng/mL]
- Schmitt, M.; Guentert, T.W. Biopharmaceutical evaluation of ketoprofen following intravenous, oral, and rectal administration in dogs. *J.Pharm.Sci.*, **1990**, *79*, 614–616 [plasma; dog; naproxen (IS); pharmacokinetics]
- Chi, S.-C.; Jun, H.W. Quantitation of ketoprofen in isopropyl myristate by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1989**, *12*, 2931–2945 [naproxen (IS)]
- Lempiainen, M.; Makela, A.L. Determination of ketoprofen by high-performance liquid chromatography from serum and urine: clinical application in children with juvenile rheumatoid arthritis. *Int.J.Clin.Pharmacol.Res.*, **1987**, *7*, 265–271
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- Hermansson, J.; Erikson, M. Direct liquid chromatographic resolution of acidic drugs using a chiral α 1-acid glycoprotein column (Enantiopac). *J.Liq.Chromatogr.*, **1986**, *9*, 621–639 [chiral; also bendroflumethiazide, disopyramide, ethotoin, hexobarbital, ibuprofen, naproxen, 2-phenoxypropionic acid, RAC 109]
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- Oka, K.; Aoshima, S.; Noguchi, M. Highly sensitive determination of ketoprofen in human serum and urine and its application to pharmacokinetic study. *J.Chromatogr.*, **1985**, *345*, 419–424
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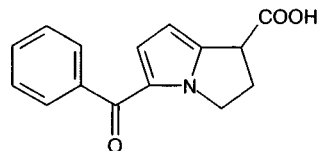
Ketorolac

Molecular formula: C₁₅H₁₃NO₃

Molecular weight: 255.3

CAS Registry No.: 74103-06-3, 74103-07-4

(tromethamine)



SAMPLE

Matrix: blood

Sample preparation: Condition a 10 × 3 C18 SPE cartridge (Analytichem) with 2 mL MeOH, 2 mL water, and 2 mL 50 mM pH 3.5 sodium acetate at 2 mL/min. 550 μL Plasma + 550 μL 0.9% NaCl, vortex vigorously, add 25 μL 10 μg/mL ketoprofen in MeOH:water 10:90, add 1 mL to the SPE cartridge at 1 mL/min, wash with 1 mL 50 mM pH 3.5 sodium acetate at 1 mL/min, wash with 1.5 mL MeOH:0.1% acetic acid 20:80 at 1.5 mL/min, elute the contents of the cartridge onto the column with mobile phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard RP-18

Column: 100 × 8 4 μm Nova-pak C18 radial pak

Mobile phase: Gradient. MeCN:0.1% acetic acid from 30:70 to 60:40 over 10 min, maintain at 60:40 for 2 min, to 100:0 over 3 min.

Flow rate: 2

Detector: UV 313 for 7.2 min then UV 258

CHROMATOGRAM

Retention time: 6.7

Internal standard: ketoprofen (8.8)

Limit of quantitation: 5 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Solà, J.; Pruñonosa, J.; Colom, H.; Peraire, C.; Obach, R. Determination of ketorolac in human plasma by high-performance liquid chromatography after automated on-line solid-phase extraction. *J.Liq.Chrom.Rel.Technol.*, **1996**, *19*, 89–99

SAMPLE

Matrix: blood

Sample preparation: Wash Amberlite XAD-2 polymeric adsorbent resin with water, acetone, and ethyl acetate and store it in ethyl acetate until use. 1 mL Blood + 100 μL 100 μg/mL cyclopentobarbital + 5 mL water + 1 g resin, vortex for 30 s or until homogeneous, let stand for 1 min, discard the supernatant, add 6 mL ethyl acetate, vortex for 1 min, centrifuge at 2000 rpm for 5 min. Remove the organic supernatant and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 100 μL MeCN, add 500 μL heptane, vortex for 5 s, centrifuge at 2000 rpm for 5 min, inject a 20 μL aliquot of the MeCN layer.

HPLC VARIABLES

Column: 250 × 4.6 Lichrospher RP-8

Mobile phase: MeCN:buffer 36:64 (Buffer was 6.8 g KH₂PO₄ and 1 mL phosphoric acid in 1 L water, pH 3.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 312

CHROMATOGRAM

Retention time: 7.0

Internal standard: cyclopentobarbital (UV 190) (4.3)

KEY WORDS

SPE

REFERENCE

Logan, B.K.; Friel, P.N.; Peterson, K.L.; Predmore, D.B. Analysis of ketorolac in postmortem blood. *J.Anal.Toxicol.*, **1995**, *19*, 61–64

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 5% zinc sulfate in water, vortex for 2 min, add 440 μ L buffer, vortex for 1 min, centrifuge at 2000 g for 10 min, inject a 100 μ L aliquot of the supernatant. (Buffer was 100 mM NaH_2PO_4 and 10 mM sodium lauryl sulfate, pH adjusted to 2.8 with phosphoric acid.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Nucleosil C18

Mobile phase: MeCN:water 35:65 containing 10 mM NaH_2PO_4 and 1 mM sodium lauryl sulfate, pH adjusted to 2.8 with phosphoric acid

Flow rate: 1.5

Injection volume: 100

Detector: UV 355

CHROMATOGRAM

Retention time: 10.3

Internal standard: ketorolac

OTHER SUBSTANCES

Extracted: tenoxicam

KEY WORDS

plasma; protect from light; ketorolac is IS

REFERENCE

Mason, J.L.; Hobbs, G.J. Simple method for the analysis of tenoxicam in human plasma using high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *665*, 410–415

SAMPLE

Matrix: blood

Sample preparation: 500 (Human) or 100 (rat) μ L plasma + 50 μ L 100 μ g/mL naproxen in MeOH + 200 μ L 600 mM sulfuric acid + 3 mL diethyl ether, vortex for 30 s, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μ L mobile phase, inject a 10-120 μ L aliquot.

HPLC VARIABLES

Guard column: 50 mm long 5 μ m silica (Phenomenex)

Column: 125 mm long Partisil 5 ODS 3 + 50 mm long chiral tert-leucine (Phenomenex)

Mobile phase: MeOH:ethyl acetate:isopropanol 50:50:2 containing 0.5 mM ammonium acetate

Flow rate: 0.8

Injection volume: 10-120

Detector: UV 313

CHROMATOGRAM

Retention time: 12 (S), 13 (R)

Internal standard: naproxen (21)

Limit of detection: <10 ng/mL

KEY WORDS

human; rat; plasma; pharmacokinetics; chiral; racemization does not occur in contrast to previous derivatization method (F. Jamali et al.; *J.Liq.Chromatogr.* 1989; 12; 1835)

REFERENCE

Vakily, M.; Corrigan, B.; Jamali, F. The problem of racemization in the stereospecific assay and pharmacokinetic evaluation of ketorolac in human and rats. *Pharm.Res.*, 1995, 12, 1652-1657

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 ng sodium tolmetin + 200 μ L 100 mM pH 4 sodium acetate, extract twice with 5 mL diethyl ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μ L water, inject an 80 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Novapak C18

Mobile phase: MeCN:1 mM pH 3 phosphoric acid 32:68

Flow rate: 1

Injection volume: 80

Detector: UV 313

CHROMATOGRAM

Retention time: 7

Internal standard: tolmetin (11)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Flores-Murrieta, F.J.; Granados-Soto, V.; Castañeda-Hernández, G.; Herrera, J.E.; Hong, E. Comparative bioavailability of two oral formulations of ketorolac tromethamine: Dolac and Exodol. *Bio-pharm.Drug Dispos.*, 1994, 15, 129-136

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 50 μ L 4 M phosphoric acid + 50 μ L 200 μ g/mL sodium S-(+)-naproxen in water + 400 μ L hexane:pentan-2-ol 90:10, mix 4 times at 1000 rpm for 30 s, centrifuge at 5000 rpm for 5 min, freeze in solid carbon dioxide for 5 min. Remove upper organic phase and add it to 150 μ L 20 mM NaOH. Mix 4 times at 1200 rpm for 30 s, centrifuge at 3000 rpm for 6 min, freeze in solid carbon dioxide for 5 min, discard upper organic phase, blot tube openings on clean filter paper. Allow to warm for 5 min to above 0°, mix at 1200 rpm for 30 s, centrifuge at 5000 rpm for 5 min, inject a 5-100 μ L aliquot.

HPLC VARIABLES**Guard column:** 10 × 3 ChromTech diol guard column**Column:** 100 × 4 ChromTech Chiral AGP-CSP**Mobile phase:** Isopropanol: 100 mM pH 5.5 NaH₂PO₄ (Use a 4 × 6 Waters Guard-Pak C18 between pump and injector.)**Flow rate:** 0.9**Injection volume:** 5-100**Detector:** UV 325

CHROMATOGRAM**Retention time:** 3.3 (R), 4.8 (S)**Internal standard:** sodium S-(+)-naproxen (6.4)**Limit of detection:** 35 ng/mL

OTHER SUBSTANCES**Simultaneous:** metabolites

KEY WORDS

plasma; recycle mobile phase; sheep

REFERENCEMills, M.H.; Mather, L.E.; Gu, X.S.; Huang, J.L. Determination of ketorolac enantiomers in plasma using enantioselective liquid chromatography on an α_1 -acid glycoprotein chiral stationary phase and ultra-violet detection. *J.Chromatogr.B*, **1994**, 658, 177-182

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Serum + 100 μ L 5% (w/v) zinc sulfate in water, vortex for 2 min, make up to 4 mL with MeOH, vortex for 2 min, centrifuge at 2000 g for 5 min, inject 100 μ L of supernatant.

HPLC VARIABLES**Guard column:** 10 μ m C18 Waters guard column**Column:** 300 × 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeCN:water 40:60 adjusted to pH 2.8 \pm 0.1 with 85% orthophosphoric acid**Flow rate:** 1.4**Injection volume:** 100**Detector:** UV 313

CHROMATOGRAM**Retention time:** 6**Limit of detection:** 10 ng/mL

KEY WORDS

serum

REFERENCEChaudhary, R.S.; Gangwal, S.S.; Jindal, K.C.; Khanna, S. Reversed-phase high-performance liquid chromatography of ketorolac and its application to bioequivalence studies in human serum. *J.Chromatogr.*, **1993**, 614, 180-184

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 50 μ L 15 μ g/mL (S)-ketoprofen in MeOH:water 1:4 + 75 μ L 2 M sulfuric acid + 100 μ L MeOH:water 1:4 + 8 mL hexane:ethyl acetate

80:20, rotary mix for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness at 55° under a stream of nitrogen. Reconstitute in 100 μ L 1.5% thionyl chloride in n-hexane (freshly prepared), vortex, heat at 80° for 30 min, cool to room temperature, add 500 μ L reagent, vortex, let stand at room temperature for 10 min, evaporate to dryness under a stream of nitrogen, add 1 mL 2 M sulfuric acid, add 5 mL ethyl acetate, mix, centrifuge. Remove the organic layer and evaporate it to dryness at 55° under a stream of nitrogen. Reconstitute in 125 μ L mobile phase, inject a 100 μ L aliquot. (Reagent was 3% (S)-1-phenylethylamine in dry dichloromethane prepared within 30 min of use.)

HPLC VARIABLES

Column: 100 \times 8 4 μ m Nova-Pak phenyl radially compressed bonded phase cartridge
Mobile phase: MeCN:20 mM sodium acetate buffer 50:50 containing 0.1% triethylamine, final pH 5.5
Flow rate: 2
Injection volume: 100
Detector: UV 310 for 8 min (derivatized ketorolac) then UV 254 (derivatized ketoprofen)

CHROMATOGRAM

Retention time: 6.5 (S), 7.2 (R)
Internal standard: (S)-ketoprofen (8.6)
Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; derivatization; chiral

REFERENCE

Hayball, P.J.; Tamblyn, J.G.; Holden, Y.; Wrobel, J. Stereoselective analysis of ketorolac in human plasma by high-performance liquid chromatography. *Chirality*, **1993**, *5*, 31–35

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL water:MeOH 9:1 + 500 μ L 300 ng/mL m-hydroxyketorolac in water:MeOH 9:1 + 100 μ L 0.5 M pH 3 sodium acetate + 5 mL diethyl ether, shake for 5 min, centrifuge at 3500 g for 5 min. Remove organic layer and add it to 3 mL hexane and 2 mL 100 mM NaOH. Shake for 5 min, centrifuge for 5 min, discard organic layer. Add 500 μ L 2 M HCl to aqueous layer, add 8 mL diethyl ether, shake for 5 min, centrifuge at 3500 g for 5 min. Remove organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue in 100 μ L mobile phase, vortex 15 s, inject a 30 μ L aliquot.

HPLC VARIABLES

Guard column: 70 \times 2.1 30-38 μ m Whatman HC Pellosil C18
Column: 250 \times 4.6 5 μ m Regis Spherisorb ODS
Mobile phase: MeCN:MeOH:20 mM pH 6 phosphate buffer containing 10 mM tetrabutyl ammonium phosphate 15:20:65 (Buffer was 88.9 parts 20 mM KH_2PO_4 + 11.1 parts 20 mM Na_2HPO_4 .)
Flow rate: 0.8
Injection volume: 30
Detector: UV 313

CHROMATOGRAM

Retention time: 7.2
Internal standard: m-hydroxyketorolac (8.3)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES**Simultaneous:** metabolites

KEY WORDSplasma

REFERENCE

Wu, A.T.; Massey, I.J. Simultaneous determination of ketorolac and its hydroxylated metabolite in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1990**, *534*, 241–246

SAMPLE**Matrix:** formulations**Sample preparation:** Add 5 mL water to 200 mg powder, sonicate for 5 min, add 15 mL MeOH, sonicate for 10 min, centrifuge an aliquot at 2500 rpm for 10 min, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 Spherisorb ODS 1**Mobile phase:** MeOH:water:acetic acid 59:40:1**Flow rate:** 1.2**Injection volume:** 25**Detector:** UV 254

CHROMATOGRAM**Retention time:** 7

OTHER SUBSTANCES**Simultaneous:** degradation products

KEY WORDSpowder

REFERENCE

Brandl, M.; Magill, A.; Rudraraju, V.; Gordon, M.S. Approaches for improving the stability of ketorolac in powder blends. *J.Pharm.Sci.*, **1995**, *84*, 1151–1153

SAMPLE**Matrix:** formulations**Sample preparation:** Add naproxen and dilute to a final concentration of about 20 µg/mL ketorolac tromethamine and 20 µg/mL naproxen.

HPLC VARIABLES**Column:** 250 × 4.6 5 µm Spherisorb ODS I**Mobile phase:** MeOH:water:acetic acid 55:44:1**Flow rate:** 1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 10.37 (ketorolac tromethamine)**Internal standard:** naproxen (14.93)

OTHER SUBSTANCES**Simultaneous:** degradation products

KEY WORDS

infusion solutions; injections; stability-indicating

REFERENCE

Floy, B.J.; Royko, C.G.; Fleitman, J.S. Compatibility of ketorolac tromethamine injection with common infusion fluids and administration sets. *Am.J.Hosp.Pharm.*, **1990**, *47*, 1097–1100

SAMPLE

Matrix: perfusate

HPLC VARIABLES

Column: 100 × 4.5 μm Chiral-AGP (Chrom-Tech)

Mobile phase: 60 mM pH 7.0 phosphate buffer

Flow rate: 1.3

Detector: UV (wavelength not specified)

CHROMATOGRAM

Retention time: 2 (R), 3.2 (S)

KEY WORDS

chiral

REFERENCE

Roy, S.D.; Chatterjee, D.J.; Manoukian, E.; Divor, A. Permeability of pure enantiomers of ketorolac through human cadaver skin. *J.Pharm.Sci.*, **1995**, *84*, 987–990

SAMPLE

Matrix: solutions

Sample preparation: Inject a 2 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Chiral AGP (ChromTech)

Mobile phase: 40 mM pH 7.0 Potassium phosphate buffer

Flow rate: 0.6

Injection volume: 2

Detector: UV 323

KEY WORDS

chiral

REFERENCE

Brandl, M.; Conley, D.; Johnson, D. Racemization of ketorolac in aqueous solution. *J.Pharm.Sci.*, **1995**, *84*, 1045–1048

SAMPLE

Matrix: solutions

Sample preparation: Acidify 5 mL solution with concentrated HCl, extract with two 5 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 1 mL d-2-octanol:toluene:sulfuric acid 2:100:0.1, heat at 40° for 19 h, neutralize with 1 mL 20 mM sodium bicarbonate. Remove the organic layer and dry it over anhydrous sodium sulfate. Remove a 200 μL aliquot and evaporate it to dryness, reconstitute with 10 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere-Si

Mobile phase: Hexane:ethyl acetate 96:42

Detector: UV 325

KEY WORDS

chiral; derivatization; normal phase

REFERENCE

Brandl, M.; Conley, D.; Johnson, D. Racemization of ketorolac in aqueous solution. *J.Pharm.Sci.*, **1995**, *84*, 1045-1048

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Ultrasphere C8

Mobile phase: MeCN:water:acetic acid 45:55:0.2

Flow rate: 1

Detector: UV 314

REFERENCE

Brandl, M.; Conley, D.; Johnson, D. Racemization of ketorolac in aqueous solution. *J.Pharm.Sci.*, **1995**, *84*, 1045-1048

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: 6.32 (A), 5.55 (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, 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, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-

dopa, 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, sulfapyrazone, 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

some 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 × 4.5 5 μm Spherisorb ODS I

Mobile phase: MeCN:water:acetic acid 44:58:1, pH 3.0

Flow rate: 1

Detector: UV 314

KEY WORDS

skin permeation; pharmacokinetics

REFERENCE

Roy, S.D.; Manoukian, E.; Combs, D. Absorption of transdermally delivered ketorolac in humans. *J.Pharm.Sci.*, **1995**, 84, 49–52

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.5 5 μm Spherisorb ODS I

Mobile phase: MeCN:water:acetic acid 44:58:1, pH 3.0

Flow rate: 1

Detector: UV 314

REFERENCE

Roy, S.D.; Manoukian, E. Transdermal delivery of ketorolac tromethamine: Permeation enhancement, device design, and pharmacokinetics in healthy humans. *J.Pharm.Sci.*, **1995**, 84, 1190–1196

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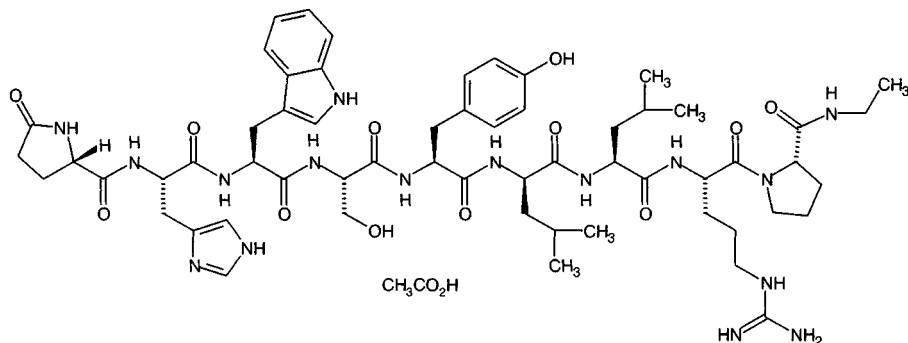
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- Hayball, P.J.; Holman, J.W.; Nation, R.L.; Massy-Westropp, R.A.; Hamon, D.P. Marked enantioselective protein binding in humans of ketorolac in vitro: elucidation of enantiomer unbound fractions following facile synthesis and direct chiral HPLC resolution of tritium-labelled ketorolac. *Chirality*, **1994**, *6*, 642–648
- Jones, D.J.; Bjorksten, A.R. Detection of ketorolac enantiomers in human plasma using enantioselective liquid chromatography. *J.Chromatogr.B*, **1994**, *661*, 165–167 [plasma; chiral; LOD 5 ng/mL; naproxen (IS)]
- Liu, H.; Wehmeyer, K.R. Supercritical fluid extraction as a sample preparation technique for the direct isolation of drugs from plasma prior to analysis. *J.Chromatogr.B*, **1994**, *657*, 206–213 [SFE; extracted flavone; dog; plasma; p-fluoroketorolac (IS); LOD <25 ng/mL]
- Roy, S.D.; Manoukian, E. Permeability of ketorolac acid and its ester analogs (prodrug) through human cadaver skin. *J.Pharm.Sci.*, **1994**, *83*, 1548–1553
- Jamali, F.; Pasutto, F.M.; Lemko, C. HPLC of ketorolac enantiomers and application to pharmacokinetics in the rat. *J.Liq.Chromatogr.*, **1989**, *12*, 1835–1850 [chiral; pharmacokinetics; rat; plasma; ketoprofen (IS); racemization occurs-see M. Vakily et al., *Pharm. Res.* 1995, *12*, 1652]

Leuprolide

Molecular formula: C₅₉H₈₄N₁₆O₁₂

Molecular weight: 1209.4

CAS Registry No.: 53714-56-0 (leuprolide), 74381-53-6 (leuprolide acetate)



SAMPLE

Matrix: formulations

Sample preparation: Mix sample with 2 mg/mL ethyl p-hydroxybenzoate in MeOH so as to give 100 µg/mL leuprolide acetate and 150 µg/mL ethyl p-hydroxybenzoate in saline, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4-4.5 5 µm octadecylsilane (IBM, Bio-Rad, or Nucleosil)

Mobile phase: MeCN:buffer 23:77 Saturate mobile phase with silica by slurring with Alltech Adsorbosil then filtering (0.4 µm). (Buffer was 87 mM (NH₄)H₂PO₄ adjusted to pH 6.5 with ammonium hydroxide.)

Flow rate: 2

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 19 (leuprolide acetate)

Internal standard: ethyl p-hydroxybenzoate (7)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

injections; stability-indicating

REFERENCE

Sutherland, J.W.; Menon, G.N. HPLC of leuprolide acetate in injectable solutions. *J.Liq.Chromatogr.*, 1987, 10, 2281-2289

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 200 × 3 Spherisorb S50DS-2

Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(\text{NH}_4)_2\text{SO}_4$. 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. Alternatively, isocratic MeCN: 0.05% phosphoric acid containing 0.5% $(\text{NH}_4)_2\text{SO}_4$ 24:76.

Flow rate: 0.5

Detector: UV 210

CHROMATOGRAM

Retention time: 20 (gradient), 13 (isocratic)

OTHER SUBSTANCES

Simultaneous: buserelin, deslorelin, gonadorelin, goserelin, 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

Levothyroxine

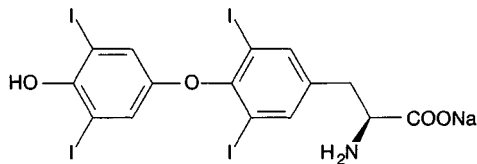
Molecular formula: C₁₅H₁₁I₄NO₄

Molecular weight: 775.9

CAS Registry No.: 55-03-8 (levothyroxine sodium),

25416-65-3 (levothyroxine sodium hydrate),

51-48-9 (levothyroxine)



SAMPLE

Matrix: blood

Sample preparation: Equilibrate a Sep-Pak silica SPE cartridge with 5 mL ethyl acetate.

1 mL Serum + 3 mL 5% trichloroacetic acid + 4 mL ethyl acetate, vortex vigorously, centrifuge at 1500 g for 5 min. Remove organic layer and repeat extraction twice with 3 mL portions of ethyl acetate. Combine extracts, evaporate to about 1.5 mL, add to the SPE cartridge. Wash with 8 mL ethyl acetate, elute with 4 mL MeOH: ammonium hydroxide (90:10). Evaporate the eluent to dryness under nitrogen, reconstitute in 100 μ L MeOH, inject.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere I.P.

Mobile phase: MeCN:buffer 35:65 (Buffer was 13.6 g sodium acetate, 1.0 g cupric sulfate pentahydrate, 0.92 g L-proline, and 0.34 g silver nitrate.)

Flow rate: 1.5

Injection volume: 100

Detector: E, Bioanalytical Systems Inc. TL-5 Kel-F glassy carbon thin-layer cell, LC-4 electronic controller, +0.78 V, 2-5 nA/V

CHROMATOGRAM

Retention time: 8

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Extracted: dextrothyroxine, triiodothyronine

KEY WORDS

serum; SPE

REFERENCE

Hay, I.D.; Annesley, T.M.; Jiang, N.S.; Gorman, C.A. Simultaneous determination of D- and L-thyroxine in human serum by liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1981**, *226*, 383-390

SAMPLE

Matrix: formulations

Sample preparation: Grind tablets containing about 1 mg levothyroxine, add 4.5 mL 0.5 mg/mL hydroxyprogesterone caproate in MeOH, add 20.5 mL 10 mM NaOH in MeOH: water 75:25, shake intermittently for 5 min, filter, discard first 5 mL filtrate, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak CN

Mobile phase: MeCN:0.1% phosphoric acid in water 35:65

Flow rate: 3

Injection volume: 25

Detector: UV 225

CHROMATOGRAM

Retention time: 5

Internal standard: hydroxyprogesterone caproate (8)

KEY WORDS

tablets

REFERENCE

Das Gupta, V.; Odom, C.; Bethea, C.; Plattenburg, J. Effect of excipients on the stability of levothyroxine sodium tablets. *J.Clin.Pharm.Ther.*, **1990**, *15*, 331–336

SAMPLE

Matrix: formulations

Sample preparation: Weigh out powder equivalent to about 65 mg thyroid, add 5 mL enzyme solution, mix well, incubate at 37° for 28 h, agitate after 4-8 h and after 20-24 h, add 2 mL deactivating solution, mix well, centrifuge at 2000 rpm for 5-10 min, if necessary filter (0.45 μ m). (The enzyme solution was about 150 protease units/mL of bacterial protease from *Streptomyces griseus* in 110 mM NaCl + 40 mM Tris buffer + 50 mM methimazole (pH adjusted to 8.4 \pm 0.05 with 6 M HCl) reducing buffer. Deactivating solution was 1:100 phosphoric acid:MeCN.)

HPLC VARIABLES

Column: 300 \times 4 μ Bondapak C18

Mobile phase: MeCN:0.5% phosphoric acid in water 28:72

Column temperature: 34

Flow rate: 1.5

Injection volume: 200

Detector: UV 225

CHROMATOGRAM

Retention time: 22

OTHER SUBSTANCES

Simultaneous: liothyronine, L-3,3',5'-triiodothyronine

KEY WORDS

tablets; powders

REFERENCE

Richheimer, S.L.; Jensen, C.B. Determination of liothyronine and levothyroxine in thyroid preparations by liquid chromatography. *J.Pharm.Sci.*, **1986**, *75*, 215–217

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, weigh out amount equivalent to about 200 μ g sodium levothyroxine, add 10 mL mobile phase, sonicate for 5 min, centrifuge. Filter (0.45 μ m, 25 mm Acrodisc CR, Gelman) the supernatant, inject a 200 μ L aliquot.

HPLC VARIABLES

Guard column: 40 \times 4 40 μ m RP 201SC pellicular (Vydac)

Column: 300 × 4 μBondapak C18

Mobile phase: MeCN:buffer 60:40 (Buffer was pH 3.0 containing 5 mM 1-octanesulfonic acid and 5 mM tetramethylammonium chloride.)

Flow rate: 2

Injection volume: 200

Detector: UV 230

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Simultaneous: liothyronine, 3,5-diiodo-L-thyronine

KEY WORDS

tablets; stability-indicating

REFERENCE

Richheimer, S.L.; Amer, T.M. Stability-indicating assay, dissolution, and content uniformity of sodium levothyroxine in tablets. *J.Pharm.Sci.*, **1983**, 72, 1349–1351

SAMPLE

Matrix: formulations

Sample preparation: Grind a tablet, add 50 μg 3,3',5'-triiodothyronine, add 20 mL solvent A, stir for 10 min, add 40 mL solvent B, stir for 30 min, filter. Remove the upper layer and wash it six times with 15 mL portions of water saturated with butanol, evaporate under vacuum at 40-42°, reconstitute in 2.5 mL 3% ammonium hydroxide in MeOH, inject an aliquot (*Anal.Lett.* 1979, 12, 1201). (Prepare the solvents by mixing 1.8 L 1-butanol, 1.35 L water and 450 mL concentrated HCl, shake vigorously for 20 min, allow to separate. The lower layer was solvent A and the upper layer was solvent B.)

HPLC VARIABLES

Guard column: 25 × 2.5 Co: Pel ODS

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:100 mM pH 5.0 ammonium acetate 50:50

Flow rate: 1.5

Detector: UV 254

CHROMATOGRAM

Retention time: 19.5

Internal standard: 3,3',5'-triiodothyronine

OTHER SUBSTANCES

Simultaneous: liothyronine

KEY WORDS

protect from light; tablets

REFERENCE

Rapaka, R.S.; Knight, P.W.; Prasad, V.K. Reversed-phase high-performance liquid chromatographic analysis of liothyronine sodium and levothyroxine sodium in tablet formulations: preliminary studies on dissolution and content uniformity. *J.Pharm.Sci.*, **1981**, 70, 131–134

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Phenomenex cyano-bonded silica

Mobile phase: MeCN:water:phosphoric acid 400:600:1

Flow rate: 1.5

Detector: UV 225

CHROMATOGRAM

Retention time: 8.6

OTHER SUBSTANCES

Simultaneous: degradation products, liothyronine

REFERENCE

Won, C.M. Kinetics of degradation of levothyroxine in aqueous solution and in solid state. *Pharm.Res.*, **1992**, *9*, 131–137

SAMPLE

Matrix: solutions

Sample preparation: Take up 1.5 mg levothyroxine 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: 9

Limit of detection: 0.05% of the D form

OTHER SUBSTANCES

Simultaneous: impurities, dextrothyroxine

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

SAMPLE

Matrix: tissue

Sample preparation: 100 μL Thyroid tissue + 200 μL MeCN, mix, centrifuge. Remove a 100 μL aliquot of the supernatant and add it to 100 μL 4 nM dabsyl chloride in MeCN, heat at 70° for 10 min, add 400 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 MeOH:25 mM pH 6.5 sodium acetate 56:44. B was MeOH. A:B from 80:20 to 35:65 over 15 min, maintain at 35:65 for 3 min, to 0:100 over 1 min, maintain at 0:100 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 436

CHROMATOGRAM

Retention time: 17.5

OTHER SUBSTANCES

Extracted: diiodothyronine (T2), liothyronine (T3)

KEY WORDS

derivatization; thyroid

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

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Fish, L.H.; Schwartz, H.L.; Cavanaugh, J.; Steffes, M.W.; Bantle, J.P.; Oppenheimer, J.H. Replacement dose, metabolism, and bioavailability of levothyroxine in the treatment of hypothyroidism. Role of triiodothyronine in pituitary feedback in humans. *N.Engl.J.Med.*, **1987**, 316, 764–770 [tablets]

Brower, J.F.; Toler, D.Y.; Reepmeyer, J.C. Determination of sodium levothyroxine in bulk, tablet, and injection formulations by high-performance liquid chromatography. *J.Pharm.Sci.*, **1984**, 73, 1315–1317

Garnick, R.L.; Burt, G.F.; Long, D.A.; Bastian, J.W.; Aldred, J.P. High-performance liquid chromatographic assay for sodium levothyroxine in tablet formulations: content uniformity applications. *J.Pharm.Sci.*, **1984**, 73, 75–77

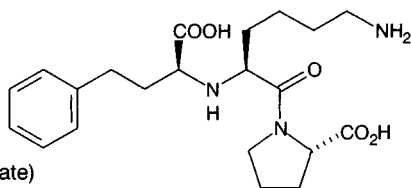
Smith, D.J.; Biesemeyer, M.; Yaciw, C. The separation and determination of liothyronine and levothyroxine in tablets by reversed-phase high performance liquid chromatography. *J.Chromatogr.Sci.*, **1981**, 19, 72–78

Lisinopril

Molecular formula: C₂₁H₃₁N₃O₅

Molecular weight: 405.5

CAS Registry No.: 76547-98-3 (anhydrous), 83915-83-7 (dihydrate)



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)) KH₂PO₄ 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: 3.50

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amitriptyline, amodiaquine, amoxapine, astemizole, atenolol, benazepril, benperidol, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenpropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephen- termine, mepivacaine, metapramine, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, pro-

guanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, secobarbital, strychnine, sulfipyrazole, sulindac, sulpride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofispam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: amisulpride, aspirin, benzocaine, carteolol, chlormezanone, codeine, mephenesin, metformin, nalorphine, naloxone, naltrexone, nizatidine, phenol, ritodrine, sotalol, sultopride

KEY WORDS

whole blood; plasma

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb 5 ODS-2

Mobile phase: n-Propanol:buffer 5:95 (Buffer was pH 3.0 phosphate buffer containing 0.4% triethylamine.)

Flow rate: 1

Detector: UV 240

CHROMATOGRAM

Retention time: 8.2

REFERENCE

Barbato, F.; Morrica, P.; Quaglia, F. Analysis of ACE inhibitor drugs by high performance liquid chromatography. *Farmaco*, **1994**, *49*, 457-460

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 µBondapak phenyl

Mobile phase: MeOH:water:85% phosphoric acid 60:40:0.05

Column temperature: 30-40

Detector: UV 215-220

OTHER SUBSTANCES

Also analyzed: enalapril

REFERENCE

Ranadive, S.A.; Chen, A.X.; Serajuddin, A.T. Relative lipophilicities and structural-pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors. *Pharm. Res.*, **1992**, *9*, 1480-1486

SAMPLE

Matrix: urine

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 20 mL 100 mM HCl. 1 mL Urine + 80 μ L 8 μ g/mL enalaprilat + 10 μ L 6 M nitric acid, vortex for 30 s, add to the SPE cartridge, wash with 20 mL 100 mM HCl, elute with 3 mL MeCN:water 10:90, elute with 6 mL water. Combine the eluates and evaporate the MeCN under a stream of air at 65°, add 25 μ L 6 M nitric acid, add this solution to the SPE cartridge, wash with 10 mL chloroform, elute with 6 mL MeOH. Evaporate the eluate to dryness under a stream of air at 65°, wash the residue with 1 mL MeCN, reconstitute with 500 μ L MeOH:chloroform 10:90, vortex for 30 s. Put this solution in another tube, evaporate to dryness, reconstitute with 100 μ L mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:THF:15 mM pH 2.9 KH₂PO₄ 6:1:1:92

Column temperature: 40

Flow rate: 1.5

Injection volume: 10

Detector: UV 206

CHROMATOGRAM

Retention time: 7.5

Internal standard: enalaprilat (10.5)

Limit of quantitation: 500 ng/mL

KEY WORDS

SPE; pharmacokinetics

REFERENCE

Wong, Y.-c.; Charles, B.G. Determination of the angiotensin-converting enzyme inhibitor lisinopril in urine using solid-phase extraction and reversed-phase high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 673, 306–310

ANNOTATED BIBLIOGRAPHY

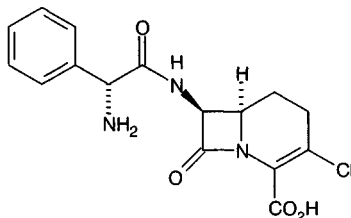
Friedman, D.I.; Amidon, G.L. Intestinal absorption mechanism of dipeptide angiotensin converting enzyme inhibitors of the lysyl-proline type: lisinopril and SQ 29,852. *J.Pharm.Sci.*, **1989**, 78, 995–998 [rat; perfusate]

Loracarbef

Molecular formula: C₁₆H₁₆ClN₃O₄

Molecular weight: 349.8

CAS Registry No.: 121961-22-6 (monohydrate)



SAMPLE

Matrix: blood, urine

Sample preparation: Plasma or serum. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH then 2 mL water, do not allow to go dry. 500 μ L Plasma or serum + 100 μ L 100 μ g/mL cephalixin in water + 50 μ L 25% acetic acid, mix, add to SPE cartridge, wash with two 1 mL portions of water, elute with 3 mL MeOH. Evaporate eluate under nitrogen, add 200 μ L mobile phase, vortex, inject a 25 μ L aliquot. Urine. Dilute 100:1 (ratio may vary depending on concentration) with water, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-18-DB

Mobile phase: MeOH:THF:buffer 16:4:80 (Buffer was 1 g sodium 1-heptanesulfonate + 15 mL triethylamine in 1 L water with the pH adjusted to 2.3 with concentrated phosphoric acid.)

Column temperature: 30

Flow rate: 1.4

Injection volume: 25-50

Detector: UV 265

CHROMATOGRAM

Retention time: 7.5

Internal standard: cephalixin (9.2)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: cefaclor, hydroxyloracarbef

Noninterfering: acetaminophen, caffeine

KEY WORDS

plasma; serum; SPE; pharmacokinetics

REFERENCE

Kovach, P.M.; Lantz, R.J.; Brier, G. High-performance liquid chromatographic determination of loracarbef, a potential metabolite, cefaclor and cephalixin in human plasma, serum and urine. *J. Chromatogr.*, **1991**, *567*, 129-139

SAMPLE

Matrix: bulk

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m C18 (YMC)

Mobile phase: Gradient. A was 6.9 g/L (NH₄)H₂PO₄ adjusted to pH 2.5 with phosphoric acid. B was MeCN:6.9 g/L (NH₄)H₂PO₄ adjusted to pH 2.5 with phosphoric acid 60:40. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 10 min.

Flow rate: 1

Detector: UV 220

CHROMATOGRAM**Retention time:** 18

OTHER SUBSTANCES**Simultaneous:** degradation products

REFERENCE

Baertschi, S.W.; Dorman, D.E.; Spangle, L.A.; Collins, M.W.; Lorenz, L.J. Formation of fluorescent pyrazine derivatives via a novel degradation pathway of the carbacephalosporin loracarbef. *J.Pharm.Biomed.Anal.*, **1995**, 13, 323–328

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 YMC A303 ODS (YMC)

Mobile phase: Gradient. A was 2.4 g/L NaH₂PO₄ adjusted to pH 2.5 with phosphoric acid. B was MeCN:2.4 g/L NaH₂PO₄ adjusted to pH 2.5 with phosphoric acid 60:40. A:B from 0:100 to 100:0 over 30 min, maintain at 100:0 for 10 min.

Flow rate: 1**Detector:** UV 210

CHROMATOGRAM**Retention time:** 15

OTHER SUBSTANCES**Simultaneous:** degradation products

REFERENCE

Skibic, M.J.; Taylor, K.W.; Oocolowitz, J.L.; Collins, M.W.; Paschal, J.W.; Lorenz, L.J.; Spangle, L.A.; Dorman, D.E.; Baertschi, S.W. Aqueous acidic degradation of the carbacephalosporin loracarbef. *J.Pharm.Sci.*, **1993**, 82, 1010–1017

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.4 Zorbax ODS**Mobile phase:** MeCN:25 mM (NH₄)H₂PO₄ 10:90**Flow rate:** 1**Detector:** UV 254

OTHER SUBSTANCES**Also analyzed:** cefaclor

REFERENCE

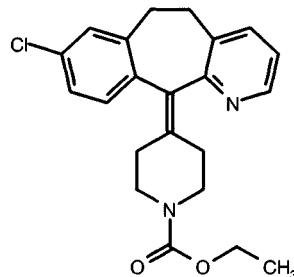
Pasini, C.E.; Indelicato, J.M. Pharmaceutical properties of loracarbef: the remarkable solution stability of an oral 1-carba-1-dethiacephalosporin antibiotic. *Pharm.Res.*, **1992**, 9, 250–254

Loratadine

Molecular formula: C₂₂H₂₃ClN₂O₂

Molecular weight: 382.9

CAS Registry No.: 79794-75-5



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)) KH₂PO₄ 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: 11.29

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demoxiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide,

moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nifedipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozone, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thio-properazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclo-marol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, triflu-peridol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: alpidem, chlorpromazine

KEY WORDS

whole blood; plasma

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 + 100 μ L IS in 0.1% phosphoric acid + 100 μ L buffer + 2 mL diethyl ether:n-hexane 75:25, shake for 10 min, centrifuge at 4000 rpm for 5 min, let stand at -27°C for 2 h. Remove the organic layer and add it to 200 μ L 12.5% phosphoric acid, shake for 10 min, centrifuge at 4000 rpm for 2 min, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 \times 4.6 7 μ m Nucleosil C18

Mobile phase: MeCN: water: (NH₄)H₂PO₄: orthophosphoric acid 110:150:1.5:8 (v/v/w/v)

Flow rate: 1.5

Injection volume: 100

Detector: F ex 290 em 460

CHROMATOGRAM

Retention time: 5

Internal standard: propyl 4-(8-chloro-5,6-dihydro-11H-benzo-[5,6]-cyclohepta-[1,2-b]pyridin-11-ylidin)-1-piperidinecarboxylate (8.5)

Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Zhong, D.; Blume, H. HPLC-Bestimmung von Loratadin und seinen aktiven Metaboliten Descarboethoxyloratadin in Humanplasma [HPLC determination of loratadine and its active metabolite descarboethoxyloratadine in human plasma]. *Pharmazie*, 1994, 49, 736-739

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: 10.90 (A), 13.25 (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, 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, fenopropfen, 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, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocaidine, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some details of plasma extraction

REFERENCE

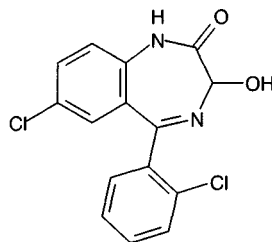
Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology. *J. Chromatogr. A*, **1995**, *692*, 103–119

Lorazepam

Molecular formula: C₁₅H₁₀Cl₂N₂O₂

Molecular weight: 321.2

CAS Registry No.: 846-49-1



SAMPLE

Matrix: blood

Sample preparation: 1-2 mL Plasma + 1 mL 1 M pH 10 bicarbonate buffer + 8 mL n-hexane:ethyl acetate 70:30, shake for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Ultrasphere ODS

Mobile phase: Gradient. A was MeCN:buffer 30:70. B was MeCN:buffer 70:30. A:B from 85:15 to 40:60 over 10 min (Waters curve no. 5), to 0:100 over 5 min (Waters curve no. 1), return to initial conditions over 10 min (Waters curve no. 1). (Buffer was 10 mM pH 3.35 NaH₂PO₄.)

Flow rate: 1

Detector: UV 229

CHROMATOGRAM

Internal standard: lorazepam

OTHER SUBSTANCES

Extracted: desmethyldiazepam, diazepam

KEY WORDS

plasma; lorazepam is IS

REFERENCE

Caraco, Y.; Tateishi, T.; Wood, A.J.J. Interethnic difference in omeprazole's inhibition of diazepam metabolism. *Clin.Pharmacol.Ther.*, **1995**, *58*, 62-72

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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: 4.19

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamazine, cyclizine, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mepredine, mephensin, mephentermine, mepivacaine, metapramine, metformin, methadone, methocarbamol, methotrexate, metipranolol, metoclopramide, mexiletine, mianserine, midazolam, minoxidil, mxclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nocardipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozone, pindolol, pipamperone, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: alprazolam, cycloguanil, ketamine, methaqualone, metoprolol, nifedipine, piroxicam, quinine, sulindac

KEY WORDS

whole blood; plasma

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: Make 1 mL serum alkaline with borate buffer, extract with cyclohexane:dichloromethane 60:40. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot

HPLC VARIABLES**Column:** C18 DB (Supelco)**Mobile phase:** MeCN:pH 2.5 phosphate buffer 37:63**Detector:** UV 254

OTHER SUBSTANCES**Extracted:** bromazepam, clobazam, diazepam, fluvoxamine, oxazepam

KEY WORDS

serum

REFERENCE

Vandenbergh, H.; MacDonald, J.C. Analysis of fluvoxamine, clobazam and other benzodiazepines on the same HPLC system (Abstract 40). *Ther.Drug Monit.*, **1995**, *17*, 393

SAMPLE**Matrix:** blood**Sample preparation:** Inject 100-200 μ L 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 \times 4 12 μ m TSK-gel G 3 PW (Tosohass); B 75 \times 4.6 Ultrasphere ODS C18 3 μ m**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

OTHER SUBSTANCES**Extracted:** alprazolam, bromazepam, chlordiazepoxide, clobazam, clorazepate, clotiazepam, desmethyloclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, loflazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam**Noninterfering:** carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid**Interfering:** clonazepam

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: 0.5 mL Plasma + 10 μ L 250 μ g/mL 1-acetamidopyrene in MeOH + 200 μ L 1 M ammonium sulfate + 800 μ L cold MeCN, vortex for 30 s, store at -20° for at least 30 min, vortex, centrifuge at 1500 g for 30 min. Remove 400 μ L of the upper organic layer and evaporate it under a stream of nitrogen. Reconstitute with 100 μ L mobile phase, vortex for 30 s, inject a 75 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak 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: 6.1

Internal standard: 1-acetamidopyrene (9.7)

Limit of detection: 0.781 ng/mL

OTHER SUBSTANCES

Simultaneous: antipyrine, indocyanine green

Noninterfering: adenosine, albuterol, alphenal, aspirin, caffeine, carbamazepine, cefazolin, cephalixin, cephalothin, cimetidine, ciprofloxacin, claforan, desipramine, enoxacin, feroxacin, 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: 1 mL Plasma + 100 μ L 1 μ g/mL diazepam in MeOH + 2 mL phosphate buffer, vortex for 30 s, add 8 mL hexane:isoamyl alcohol 95:5, shake gently by hand for 10 min, vortex for 1 min, centrifuge at 400 g at 4° for 10 min. Remove organic layer and add it to 2 mL 6 M HCl, shake for 10 min, vortex for 1 min, centrifuge at 400 g for 10 min. Remove the aqueous layer and slowly add about 2 mL 6 M NaOH to it to achieve a pH greater than 7.0, add 2 mL phosphate buffer, vortex for 10 s, add 8 mL hexane:isoamyl alcohol 95:5, shake for 10 min, vortex for 1 min, centrifuge at 400 g for 10 min. Remove the organic layer and evaporate it to dryness at 40° with nitrogen, rinse residue from sides with hexane:isoamyl alcohol 95:5, again dry with nitrogen, take up residue in 40 μ L MeOH, inject a 20-40 μ L aliquot. (Phosphate buffer was 136.1 g KH_2PO_4 in 1 L water, adjusted to pH 7 with 1 M K_2HPO_4 .)

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:MeCN:10 mM sodium acetate 40:12.5:47.5, at pH 4.6

Flow rate: 2.2

Injection volume: 20-40

Detector: UV 254

CHROMATOGRAM

Retention time: 5.2

Internal standard: diazepam (9.2)

Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: midazolam, flunitrazepam, clonazepam, flurazepam, temazepam, nitrazepam

Interfering: oxazepam

KEY WORDS

plasma

REFERENCE

Egan, J.M.; Abernethy, D.R. Lorazepam analysis using liquid chromatography: improved sensitivity for single-dose pharmacokinetic studies. *J.Chromatogr.*, **1986**, *380*, 196-201

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 μ L of 100 μ g/mL phenacetin and 3 μ g/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 μ L MeCN:0.1% pH 3 sodium phosphate buffer 30:70, inject an aliquot.

HPLC VARIABLES

Guard column: 23 \times 3.9 37-50 μ m μ Bondapak phenyl

Column: 300 \times 3.9 10 μ m μ Bondapak 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 229

CHROMATOGRAM

Retention time: 15.11

Internal standard: flunitrazepam (17.90)

Limit of quantitation: 10.5 ng/mL

OTHER SUBSTANCES

Simultaneous: antipyrine (at 254 nm)

Noninterfering: acetaminophen, allopurinol, indocyanine green, sulfamethoxazole, trimethoprim

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, CSF, gastric fluid, urine

Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m 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: 12.57

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, 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: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m cyano

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 220

CHROMATOGRAM

Retention time: 8.72

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: formulations

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Spherisorb ODS-1

Mobile phase: MeOH:50 mM (NH₄)H₂PO₄ 50:50, adjusted to pH 7.22

Flow rate: 1.3 for 12 min then 2.1 for 14 min

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

stability-indicating; 5% dextrose; saline; injections

REFERENCE

Mancano, M.A.; Boullata, J.I.; Gelone, S.P.; Zitterman, R.E.; Borenstein, M.R. Availability of lorazepam after simulated administration from glass and polyvinyl chloride containers. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2213–2216

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water.

HPLC VARIABLES

Column: Waters C18 column (PN 86344)

Mobile phase: MeOH:50 mM (NH₄)H₂PO₄ 50:50, adjusted to pH 6.5 with ammonium hydroxide

Flow rate: 2

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 21.1

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:** Inject an aliquot directly.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Vydac 208TP54 C8**Mobile phase:** MeOH:50 mM (NH₄)H₂PO₄ 57:43, pH adjusted to 6.5 with ammonium hydroxide**Flow rate:** 1**Detector:** UV 254

CHROMATOGRAM**Retention time:** 6.1

KEY WORDS

injections; saline; 5% dextrose; lactated Ringer's; stability-indicating

REFERENCETrissel, L.A.; Pearson, S.D. Storage of lorazepam in three injectable solutions in polyvinyl chloride and polyolefin bags. *Am.J.Hosp.Pharm.*, **1994**, *51*, 368–372

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:** MeOH:THF:water 50:5:50**Flow rate:** 3**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES**Simultaneous:** diazepam, thiopental

KEY WORDS

injections; saline

REFERENCEMartens, 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:** 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: 6

OTHER SUBSTANCES

Simultaneous: bromazepam, chlordiazepoxide, clobazam, clorazepate, diazepam, flunitrazepam, 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: 6.3

OTHER SUBSTANCES

Simultaneous: bromazepam, chlordiazepoxide, clobazam, clorazepate, diazepam, flunitrazepam, 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: 6.14 (A), 5.78 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atro-

pine, 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, fenopropfen, 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, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, sulfapyrazone, 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

some 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 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 \times 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 \times 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 \times 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)

Mobile phase: EtOH:50 mM pH 5.5 KH₂PO₄ 5:95

Flow rate: 0.8

Injection volume: 20

Detector: UV

CHROMATOGRAM**Retention time:** k' 4.02

OTHER SUBSTANCES**Simultaneous:** bepridil, manidipine, nicardipine**Interfering:** oxazepam

KEY WORDSchiral; $\alpha = 1.63$

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

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica**Mobile phase:** Heptane:isopropanol 90:10**Flow rate:** 1**Injection volume:** 1000**Detector:** UV 254

CHROMATOGRAM**Retention time:** k' 9.84

KEY WORDSchiral; $\alpha 1.64$

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:** solutions**Sample preparation:** Dissolve the compound, S-trolox methyl ether (Fluka), dicyclohexylcarbodiimide, and 4-dimethylaminopyridine in dichloromethane, stir at room temperature for 1 h, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES**Column:** 300 × 0.32 5 μm LiChrosorb Diol**Mobile phase:** Carbon dioxide:MeOH 91.5:8.5**Column temperature:** 80**Injection volume:** 0.2**Detector:** UV 254

CHROMATOGRAM**Retention time:** 50.2 (second peak)

KEY WORDS

derivatization; subcritical fluid chromatography; chiral; density of mobile phase 0.62 g/mL; resolution (R_s) 1.2

REFERENCE

Almquist, S.R.; Petersson, P.; Walther, W.; Markides, K.E. Direct and indirect approaches to enantiomeric separation of benzodiazepines using micro column techniques. *J.Chromatogr.A*, **1994**, 679, 139-146

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: 8.5

OTHER SUBSTANCES

Simultaneous: bromazepam, chlorazepate, chlordiazepoxide, clobazam, diazepam, flunitrazepam, nitrazepam, oxazepam, tofisopam

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, 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, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropofen, 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, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyriline, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 Chiralpak OD (Daicel)

Mobile phase: Carbon dioxide: MeCN: EtOH: diethylamine 69.5: 15: 15: 0.5

Column temperature: 30

Flow rate: 2

Detector: UV 220

CHROMATOGRAM

Retention time: 5.2, 7 (enantiomers)

KEY WORDS

SFC; chiral; pressure 200 bar

REFERENCE

Kot, A.; Sandra, P.; Venema, A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs. *J.Chromatogr.Sci.*, **1994**, *32*, 439–448

ANNOTATED BIBLIOGRAPHY

Haginaka, J.; Kanasugi, N. Enantioselectivity of bovine serum albumin-bonded columns produced with isolated protein fragments. *J.Chromatogr.A*, **1995**, *694*, 71–80 [chiral; also benzoin, clorazepate, fenopropfen, flurbiprofen, ibuprofen, ketoprofen, lormetazepam, oxazepam, pranoprofen, temazepam, warfarin]

Ficarra, R.; Ficarra, P.; Tommasini, S.; Carulli, M.; Costantino, D.; Calabrò, M.L. Chromatographic investigations of brotizolam. *Farmaco*, **1994**, *49*, 437–440 [simultaneous brotizolam; tablets; lorazepam is IS]

Herman, R.J.; Chaudhary, A.; Szakacs, C.B. Disposition of lorazepam in Gilbert's syndrome: Effects of fasting, feeding, and enterohepatic circulation. *J.Clin.Pharmacol.*, **1994**, *34*, 978–984 [plasma; urine]

Fujima, H.; Wada, H.; Miwa, T.; Haginaka, J. Chiral separation of lorazepam on ovomucoid-bonded columns: Peak coalescence due to racemization. *J.Liq.Chromatogr.*, **1993**, *16*, 879–891 [chiral; column temp 7]

Kondo, T.; Buss, D.C.; Routledge, P.A. A method for rapid determination of lorazepam by high-performance liquid chromatography. *Ther.Drug Monit.*, **1993**, *15*, 35–38

Gunawan, S.; Walton, N.Y.; Treiman, D.M. Analysis of lorazepam in rat brain using liquid/liquid and solid-phase extraction in combination with high performance liquid chromatography. *Bio-med.Chromatogr.*, **1990**, *4*, 168–170 [SPE]

Noggle, F.T., Jr.; Clark, C.R.; DeRuiter, J. Liquid chromatographic separation of some common benzodiazepines and their metabolites. *J.Liq.Chromatogr.*, **1990**, *13*, 4005–4021 [also alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, demoxepam, diazepam, estazolam, fludiazepam, flunitrazepam, flurazepam, halazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam, triazolam]

Gunawan, S.; Treiman, D.M. Determination of lorazepam in plasma of patients during status epilepticus by high-performance liquid chromatography. *Ther.Drug Monit.*, **1988**, *10*, 172–176

Pietrogrande, M.C.; Dondi, F.; Blo, G.; Borea, P.A.; Bigli, C. Retention behavior of benzodiazepines in normal-phase HPLC. Silica, cyano, and amino phases comparison. *J.Liq.Chromatogr.*, **1988**, *11*, 1313–1333 [also metabolites diazepam, medazepam, methyl lorazepam, oxazepam, prazepam, temazepam]

Wong, S.H.Y.; McHugh, S.L.; Dolan, J.; Cohen, K.A. Tricyclic antidepressant analysis by reversed-phase liquid chromatography using phenyl columns. *J.Liq.Chromatogr.*, **1986**, *9*, 2511–2538 [also acetaminophen, amitriptyline, amobarbital, amoxapine, barbital, chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, codeine, desipramine, desmethyldoxepin, diazepam, doxepin, fluphenazine, flurazepam, glutethimide, hydroxyamoxapine, imipramine, maprotiline, meperidine, metabolites, nortriptyline, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, propoxyphene, protriptyline, secobarbital, thioridazine, trazodone]

Pietrogrande, M.C.; Bigli, C.; Borea, P.A.; Barbaro, A.M.; Guerra, M.C.; Biagi, G.L. Relationship between log k' values of benzodiazepines and composition of the mobile phase. *J.Liq.Chromatogr.*, **1985**, *8*, 1711–1729 [also carbenicillin, chlordiazepoxide, diazepam, dicloxacillin, flurazepam, medazepam, methyl lorazepam, methyl lorazepam, nitrazepam, oxazepam, prazepam, temazepam, testosterone]

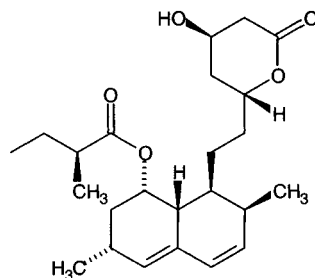
Walmsley, L.M.; Chasseaud, L.F. High-performance liquid chromatographic determination of lorazepam in monkey plasma. *J.Chromatogr.*, **1981**, *226*, 155–163

Lovastatin

Molecular formula: C₂₄H₃₆O₅

Molecular weight: 404.6

CAS Registry No.: 75330-75-5



SAMPLE

Matrix: bile, blood

Sample preparation: Prepare a 1 mL Bond-Elut C2 SPE cartridge by washing with 1 mL MeOH and 1 mL buffer. Plasma. 500 μ L Plasma + 500 μ L buffer, keep in an ice water mixture, add 100 μ L MeCN:water 60:40, add 50 μ L 5 μ g/mL methyl mevinolinic acid in MeCN:water 60:40, mix, add to the SPE cartridge, wash twice with 1 mL buffer, wash twice with 1 mL MeCN:buffer 20:80, elute with 400 μ L MeCN:water 75:25, inject a 30 μ L aliquot of eluant. Bile. Inject a 10 μ L aliquot directly. (Buffer was 100 mM pH 7.2 potassium phosphate buffer.)

HPLC VARIABLES

Column: 50 \times 4.6 3 μ m Sepralyte C18

Mobile phase: MeCN:50 mM ammonium phosphate + 10 mM phosphoric acid. Isocratic 50:50 (plasma). Gradient 20:80 to 75:25 over 10 min (bile).

Flow rate: 1.5

Injection volume: 30 (plasma), 10 (bile)

Detector: UV 238

CHROMATOGRAM

Retention time: 4.95

Internal standard: methyl mevinolinic acid (3.50)

Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Simultaneous: mevinolinic acid

KEY WORDS

plasma; human; rat; dog; SPE

REFERENCE

Stubbs, R.J.; Schwartz, M.; Bayne, W.F. Determination of mevinolin and mevinolinic acid in plasma and bile by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *383*, 438-443

SAMPLE

Matrix: bile, microsomal incubations

Sample preparation: Microsomal incubations. Extract 10 mL microsomal incubations with 30 mL ethyl acetate:acetone 2:1, centrifuge. Remove organic layer and dry it over anhydrous sodium sulfate, filter, evaporate under vacuum, reconstitute in n-propanol, inject an aliquot. Bile. Acidify to pH 3.5 with 20 mM formic acid, extract with toluene: n-propanol 85:15, extract with n-butanol saturated with water, combine extracts, evaporate under vacuum, reconstitute in n-propanol, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 10 μ Bondapak ODS

Mobile phase: Gradient. MeCN:water from 30:70 to 90:10 over 30 min.

Flow rate: 3

Detector: UV 238

CHROMATOGRAM

Retention time: 26

OTHER SUBSTANCES

Simultaneous: metabolites

REFERENCE

Vyas, K.P.; Kari, P.H.; Pitzenberger, S.M.; Halpin, R.A.; Ramjit, H.G.; Arison, B.; Murphy, J.S.; Hoffman, W.F.; Schwartz, M.S.; Ulm, E.H. Biotransformation of lovastatin. I. Structure elucidation of *in vitro* and *in vivo* metabolites in the rat and mouse. *Drug Metab.Dispos.*, **1990**, *18*, 203–211

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 1 mL acetone + 2 mL ethyl acetate, extract, centrifuge. Remove the organic layer and dry it over anhydrous sodium sulfate, concentrate under a stream of nitrogen. Reconstitute in 200 μ L n-propanol, inject 20-50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: Gradient. MeCN:5 mM formic acid from 30:70 to 90:10 over 30 min.

Flow rate: 1

Injection volume: 20-50

Detector: UV 238

CHROMATOGRAM

Retention time: 25

OTHER SUBSTANCES

Simultaneous: metabolites

REFERENCE

Vyas, K.P.; Kari, P.H.; Prakash, S.R.; Duggan, D.E. Biotransformation of lovastatin. II. In vitro metabolism by rat and mouse liver microsomes and involvement of cytochrome P-450 in dehydrogenation of lovastatin. *Drug Metab.Dispos.*, **1990**, *18*, 218–222

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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: 10.49 (A), 14.79 (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, 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, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, 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, sulfapyrazone, 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

some 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: Centrifuge at 2000 rpm, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS

Mobile phase: MeCN: water: triethylamine: glacial acetic acid 500:500:1:1

Column temperature: 30

Flow rate: 2

Detector: UV 238

CHROMATOGRAM

Retention time: 14.4 (4.4 hydroxy acid form)

Limit of detection: 10 ng/mL

Limit of quantitation: 25 ng/mL

REFERENCE

Serajuddin, A.T.; Ranadive, S.A.; Mahoney, E.M. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin. *J.Pharm.Sci.*, **1991**, *80*, 830–834

SAMPLE

Matrix: tissue

Sample preparation: Homogenize in 4 mL water, add 4 mL ice-cold acetone, centrifuge at 3000 rpm for 15 min, extract twice with an equal volume of hexanes. Evaporate extracts under vacuum, reconstitute in n-propanol, inject an aliquot.

HPLC VARIABLES

Column: 300 × 7 Hamilton PRP-1

Mobile phase: Gradient. MeCN:5 mM formic acid from 30:70 to 90:10 over 30 min.

Flow rate: 3

Detector: UV 238

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

liver

REFERENCE

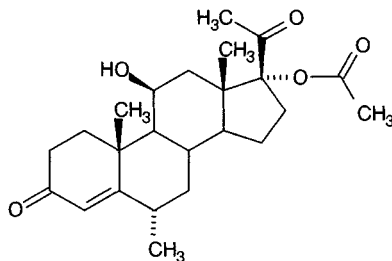
Vyas, K.P.; Kari, P.H.; Pitzenberger, S.M.; Halpin, R.A.; Ramjit, H.G.; Arison, B.; Murphy, J.S.; Hoffman, W.F.; Schwartz, M.S.; Ulm, E.H. Biotransformation of lovastatin. I. Structure elucidation of *in vitro* and *in vivo* metabolites in the rat and mouse. *Drug Metab.Dispos.*, **1990**, *18*, 203–211

Medroxyprogesterone Acetate

Molecular formula: C₂₄H₃₄O₄

Molecular weight: 386.5

CAS Registry No.: 71-58-9 (medroxyprogesterone acetate),
520-85-4 (medroxyprogesterone)



SAMPLE

Matrix: blood

Sample preparation: Extract 0.1-1 mL plasma twice with 40 volumes of diethyl ether, evaporate the organic solvent, dissolve the residue in 100 μ L MeOH:water 80:20, inject a 30 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Beckman RP-ODS

Mobile phase: Gradient. MeCN:water from 40:60 to 70:30 over 20 min, then at 70:30 for 5 min.

Injection volume: 30

Detector: UV 238

CHROMATOGRAM

Retention time: 20.5

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

S Sturm, G.; Haberlein, H.; Bauer, T.; Plaum, T.; Stalker, D.J. Mass spectrometric and high-performance liquid chromatographic studies of medroxyprogesterone acetate metabolites in human plasma. *J.Chromatogr.*, **1991**, *562*, 351-362

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 1 mL 0.5 μ g/mL 16 β -methylprogesterone in 200 mM pH 7.0 phosphate buffer, vortex for 3 s, add 7 mL hexane, mix on a rolling mixer for 30 min. Remove the hexane layer and evaporate it to dryness at 30° under nitrogen. Dissolve residue in 200 μ L mobile phase, inject a 150 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb 5-ODS2

Mobile phase: MeOH: 20 mM pH 4 acetate buffer 79:21

Flow rate: 1.5

Injection volume: 150

Detector: UV 240

CHROMATOGRAM

Retention time: 5.3

Internal standard: 16 β -methylprogesterone (9.0)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: aldosterone, androstenedione, corticosterone, cortisone, estradiol, 17 α -hydroxyprogesterone, progesterone, testosterone

Noninterfering: cholesterol

Interfering: lignocaine

KEY WORDS

plasma

REFERENCE

Read, J.; Mould, G.; Stevenson, D. Simple high-performance liquid chromatographic method for the determination of medroxyprogesterone acetate in human plasma. *J.Chromatogr.*, **1985**, *341*, 437–444

SAMPLE

Matrix: formulations

Sample preparation: Grind tablets to a fine powder, weigh out an amount equivalent to about 10 mg medroxyprogesterone acetate, add 10 mL MeOH, sonicate for 5 min, dilute to 50 mL with MeOH. Dilute 25 mL of this solution to 50 mL with MeOH, filter (0.45 μ m), inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m Novapak C18

Mobile phase: MeOH:10 mM (NH₄)₂HPO₄ 80:20, adjust pH to 7.2 \pm 0.1 with 85% phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 6.7

KEY WORDS

tablets; stability-indicating

REFERENCE

Fatmi, A.A.; Williams, G.V.; Hickson, E.A. Liquid chromatographic determination of medroxyprogesterone acetate in tablets. *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 528–530

SAMPLE

Matrix: formulations

Sample preparation: Suspensions. Dilute 2 mL suspension to 1 L with EtOH, remove a 2.5 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. Tablets. Grind tablets to a fine powder, stir with 50 mL EtOH, make up to 100 mL with EtOH, filter (Whatman No. 1 paper), reject the first 20 mL of the filtrate. Mix 2 mL of the filtrate with 1 mL 1 mg/mL hydrocortisone in EtOH, make this mixture up to 50 mL with EtOH, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 4 μ Bondapak CN

Mobile phase: MeOH:20 mM KH₂PO₄ 30:70

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM**Retention time:** 7.5**Internal standard:** hydrocortisone (2.5)

OTHER SUBSTANCES**Simultaneous:** benzyl benzoate, progesterone**Noninterfering:** methylcellulose, myristyl-gamma-picolinium chloride, polyethylene glycol 4000, thimerosal

REFERENCEDas 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:** 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' 6.712 (medroxyprogesterone acetate), k' 3.430 (medroxyprogesterone)

OTHER SUBSTANCES**Also analyzed:** androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), 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)

REFERENCESadlej-Sosnowska, N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors. *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330

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:** 10 (for medroxyprogesterone)

OTHER SUBSTANCES**Simultaneous:** dienestrol, diethylstilbestrol, hexestrol, 17α-methyltestosterone, nandrolone, trenbolone, zeranol

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

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

CHROMATOGRAM

Retention time: k' 3.12

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: 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; MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM

Retention time: 13.7

Limit of detection: 100 ppb

OTHER SUBSTANCES

Extracted: dexamethasone, diethylstilbestrol, 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.Chrom.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: 14 (for medroxyprogesterone)

Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: trans-diethylstilbestrol, 17α-methyltestosterone, nandrolone, 17β-trenbolone, zeranol

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

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Mould, G.P.; Read, J.; Edwards, D.; Bye, A. A comparison of the high-performance liquid chromatography and RIA measurement of medroxyprogesterone acetate. *J.Pharm.Biomed.Anal.*, **1989**, *7*, 119–122

Rees, H.D.; Bonsall, R.W.; Michael, R.P. Pre-optic and hypothalamic neurons accumulate [³H]medroxyprogesterone acetate in male cynomolgus monkeys. *Life Sci.*, **1986**, *39*, 1353–1359 [brain; tissue; UV detection]

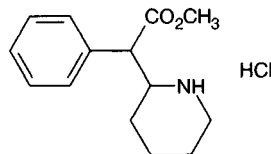
Milano, G.; Carle, G.; Renee, N.; Boubilil, J.L.; Namer, M. Determination of medroxyprogesterone acetate in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *232*, 413–417

Methylphenidate

Molecular formula: C₁₄H₁₉NO₂

Molecular weight: 233.3

CAS Registry No.: 113-45-1 (methylphenidate), 298-59-9
(methylphenidate hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 40 μ L 1 μ g/mL ethylphenidate + 1 mL 200 mM pH 9.1 carbonate buffer + 5 mL hexane:ethyl acetate 75:25, mix for 10 min, centrifuge at 1000 g for 5 min. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μ L MeCN, inject a 75 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:0.07% triethylamine 35:65, adjust pH to 3.4 with concentrated phosphoric acid.

Flow rate: 1.5

Injection volume: 75

Detector: UV 192

CHROMATOGRAM

Retention time: 3.2

Internal standard: ethylphenidate (Prepare ethylphenidate by refluxing methylphenidate in acidic EtOH for 72 h.) (4.6)

Limit of detection: 2.5 ng/mL

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, caffeine, cimetidine, diazepam, diltiazem, doxepin, flurazepam, nifedipine, procainamide, quinidine, theophylline, verapamil

KEY WORDS

plasma

REFERENCE

Lalande, M.; Wilson, D.L.; McGilveray, I.J. HPLC determination of methylphenidate in human plasma. *J.Liq.Chromatogr.*, **1987**, *10*, 2257-2264

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 NaH₂PO₄ containing 0.5% NaF. Add 500 μ L brain homogenate or 500 μ L plasma, saliva, or urine containing 15 μ L saturated NaF solution to 75 μ L 150 μ g/mL IS, add 50 μ L 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 μ L 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 μ L 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 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m Brownlee RP-8

Column: 250 \times 4.6 5 μ m 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: 4.6

Internal standard: 2 β -carbomethoxy-3 β -(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, clozapine, cocaine, gepirone, pentazocine, pseudococaine

Simultaneous: acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, bupropion, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hyderygine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaqualone, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylcegonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428

Noninterfering: amitriptyline, benzotropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobamate, 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: bulk

Sample preparation: Dissolve 100 mg bulk drug in 5 mL buffer, extract with 5 mL chloroform, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 35 $^\circ$, reconstitute the residue in 1 mL chloroform, inject a 20 μ L aliquot. (Prepare buffer by adjusting the pH of 100 mM sodium bicarbonate to 10 with 100 mM NaOH.)

HPLC VARIABLES

Column: 100 \times 2.1 Sil-X (Perkin-Elmer)

Mobile phase: Chloroform:cyclohexane:EtOH:concentrated ammonium hydroxide 85:13.5:1.5:0.5

Flow rate: 0.4

Injection volume: 20

Detector: UV 254

CHROMATOGRAM**Retention time:** 20**Limit of detection:** 0.1%

KEY WORDS

normal phase; separation of diastereomers

REFERENCE

Padmanabhan, G.R.; Fogel, J.; Mollica, J.A.; O'Connor, J.M.; Strusz, R. Application of high pressure liquid chromatography to the determination of diastereomer in methylphenidate hydrochloride. *J.Liq.Chromatogr.*, **1980**, *3*, 1079–1085

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 Bio-Sil C18 HL 90-5 (Bio-Rad)**Mobile phase:** Gradient. MeCN:80 mM phosphoric acid from 0:100 to 70:30 over 10 min.**Flow rate:** 1**Injection volume:** 10**Detector:** UV 200

CHROMATOGRAM**Retention time:** 14

OTHER SUBSTANCES

Simultaneous: amphetamine, cocaine, hydroxyamphetamine, nylidrin, oxymetazoline, phendimetrazine, tetrahydrozoline

REFERENCE

Life Science Research Products, Bio-Rad, Hercules CA, 1995, p. 76

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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:** 8.61 (A), 4.65 (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, cimet-

dine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diffunisal, 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, methyl dopa, 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, sulfapyrazone, 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

some 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 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, diffunisal, 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, fenopropfen, 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, isocarboxazid, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methylidopa, methyl dopamine, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, pyromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 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: 3.495

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylcegonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methyprylon, N-norcocaine, 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: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 1.80

OTHER SUBSTANCES

Simultaneous: acetylcodeine, amphetamine, benzphetamine, benzylmorphine, bromo-STP, buprenorphine, chlorphentermine, codeine, codeine-N-oxide, dextromoramide, dextropropoxyphene, diamorphine, diethylpropion, dihydrocodeine, dihydromorphine, dimethylamphetamine, dipipanone, ephedrine, epinephrine, ethoheptazine, ethylmorphine, etorphine, fencamfamin, fenfluramine, fentanyl, hydrocodone, 4-hydroxyamphetamine, levorphanol, mazindol, meperidine, mephentermine, mescaline, methadone, methamphetamine, methylenedioxyamphetamine, methylephedrine, monoacetylmorphine, morphine, morphine-3-glucuronide, morphine-N-oxide, naloxone, norcodeine, norlevorphanol, normetanephrine, normethadone, normorphine, norpethidine, norpseudoephedrine, noscapine, oxycodone, papaverine, pemoline, pentazocine, 2-phenethylamine, phenoperidine, phentermine, phenylephrine, phenylpropanolamine, pholcodeine, pipradol, piritramide, prolintane, pseudoephedrine, STP, thebacon, thebaine, trimethoxyamphetamine, tyramine

Noninterfering: dopamine, levodopa, methyl dopa, methyl dopate, norepinephrine

Interfering: caffeine, fenethyline, hydroxypethidine, levallorphan, nalorphine, norpipanone, phenazocine, phendimetrazine, phenelzine, tranlycypromine

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: μ Bondapak C18 (A), or μ Bondapak alkyl phenyl (B), or μ Bondapak CN (C)

Mobile phase: MeOH:water:acetic acid 20:79:1 containing 5 mM methanesulfonic acid

CHROMATOGRAM

Retention time: k' 9.36 (A), k' 8.95 (B), k' 1.82 (C)

OTHER SUBSTANCES

Also analyzed: acetaminophen, acetylcodeine, acetylmorphine, aminopyrene, aminopyrine, amobarbital, amphetamine, antipyrine, benzocaine, butabarbital, caffeine, cocaine, codeine, diamorphine, diazepam, diethylpropion, DMT, ephedrine, glutethimide, Lampa, lidocaine, LSD, MDA, mecloqualone, mescaline, methamphetamine, methapyrilene, methaqualone, methpyrilene, morphine, narcotine, papaverine, PCP, pentobarbital, phencyclidine, phendimetrazine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, procaine, quinidine, quinine, secobarbital, strychnine, TCP, tetracaine, thebaine, theophylline

REFERENCE

Lurie, I.S.; Demchuk, S.M. Optimization of a reverse phase ion-pair chromatographic separation for drugs of forensic interest. Part II—Factors effecting selectivity. *J.Liq.Chromatogr.*, **1981**, *4*, 357–374

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 200 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: 200

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. PTFE tubing in a three-dimensional figure eight configuration. The irradiation apparatus was maintained at 0–5° using an ice bath.

CHROMATOGRAM

Retention time: 4

Limit of detection: 750 ppb

OTHER SUBSTANCES

Simultaneous: chlordiazepoxide, cocaine, nitrazepam, phenobarbital

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

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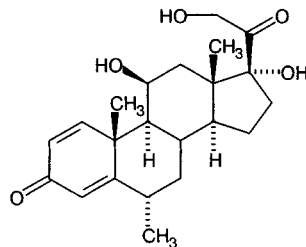
Schill, G.; Wainer, I.W.; Barkan, S.A. Chiral separation of cationic drugs on an α1-acid glycoprotein bonded stationary phase. *J.Liq.Chromatogr.*, **1986**, *9*, 641–666 [chiral; also atropine, bromdiphenhydramine, brompheniramine, bupivacaine, butorphanol, carbinoxamine, chlorpheniramine, clidinium, cocaine, cyclopentolate, dimethindene, dipiperidone, disopyramide, doxylamine, ephedrine, homatropine, labetalol A, labetalol B, mepensolate, mepivacaine, methadone, methorphan, methylatropine, methylhomatropine, metoprolol, nadolol, nadolol A, nadolol B, oxprenolol, oxyphenyclimine, phenmetrazine, phenoxybenzamine, promethazine, pronethalol, propoxyphene, propranolol, pseudoephedrine, terbutaline, tocinide, tridihexethyl]

Methylprednisolone

Molecular formula: C₂₂H₃₀O₅

Molecular weight: 374.5

CAS Registry No.: 83-43-2, 53-36-1 (acetate), 5015-36-1 (sodium phosphate), 2375-03-3 (sodium succinate), 2921-57-5 (hemisuccinate), 90350-40-6 (sulfate)



SAMPLE

Matrix: blood

Sample preparation: Extract 1 mL plasma containing dexamethasone with 12 mL dichloromethane. Remove the organic phase and wash it with 2 mL 100 mM NaOH, wash with 1 mL water, dry over 1 g anhydrous sodium sulfate. Evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb silica

Mobile phase: Hexane:dichloromethane:EtOH:glacial acetic acid 26:69:3.4:2

Flow rate: 0.75

Detector: UV 254

CHROMATOGRAM

Retention time: 7.4

Internal standard: dexamethasone (5.1)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: hydrocortisone, prednisolone

KEY WORDS

plasma; pharmacokinetics; normal phase

REFERENCE

Möllmann, H.; Hochhaus, G.; Rohatagi, S.; Barth, J.; Derendorf, H. Pharmacokinetic/pharmacodynamic evaluation of deflazacort in comparison to methylprednisolone and prednisolone. *Pharm.Res.*, **1995**, *12*, 1096-1100

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 NaH₂PO₄ 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; UV 256; UV 343

CHROMATOGRAM

Retention time: 18.70 (methylprednisolone), 21.36 (methylprednisolone acetate)

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, dexamethasone, fluen-drenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17 δ -hydroxyprogesterone, meprednisone, paramethasone, prednisolone, prednisone, progesterone, triamcinolone

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE

Volin, P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, 666, 347–353

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 500 ng fluorometholone + 5 mL hexane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min, discard the hexane layer, add 8 mL dichloromethane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min, repeat extraction with 8 mL dichloromethane. Combine the dichloromethane layers and add 300 mg anhydrous sodium sulfate, shake horizontally at 200 cycles/min for 5 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L mobile phase, add 2 mL hexane, vortex for 1 min, centrifuge at 1000 g for 5 min, discard the hexane layer, inject a 100 μ L aliquot of the mobile phase layer.

HPLC VARIABLES

Guard column: 30 \times 4.6 5 μ m Spheri-5 RP-18

Column: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:water:glacial acetic acid 33:62:5

Flow rate: 1

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 8.07 (methylprednisolone), 21.80 (methylprednisolone acetate)

Internal standard: fluorometholone (14.55)

Limit of detection: 1 ng/mL

Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Hopkins, N.K.; Wagner, C.M.; Brisson, J.; Addison, T.E. Validation of the simultaneous determination of methylprednisolone and methylprednisolone acetate in human plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1992**, 577, 87–93

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 100 μ L 3 M sulfuric acid + 50 μ L 6 μ g/mL prednisone in MeCN:MeOH 50:50, mix, add 15 mL hexane:ethyl acetate 50:50, shake for 20 min, centrifuge, freeze at -70° . Remove the organic layer and add it to 1 mL 1 M nitric acid, shake, freeze. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 30° , reconstitute the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES**Guard column:** 12.5 \times 4.5 μ m Zorbax SIL**Column:** three 80 \times 4.5 μ m Zorbax SIL Reliance 5 columns in series**Mobile phase:** Dichloromethane:hexane:EtOH:glacial acetic acid 69:26:2.3:1 (Pass the mobile phase through a 70 \times 6 37-53 μ m HC-Pellocil (Whatman) column.)**Flow rate:** 2**Detector:** UV 254

CHROMATOGRAM**Retention time:** 12 (methylprednisolone hemisuccinate), 19 (methylprednisolone)**Internal standard:** prednisone (9)**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** hydrocortisone

KEY WORDS

plasma; pharmacokinetics; normal phase

REFERENCEKong, A.-N.; Slaughter, R.L.; Jusko, W.J. Simultaneous analysis of methylprednisolone hemisuccinate, cortisol and methylprednisolone by normal-phase high-performance liquid chromatography in human plasma. *J.Chromatogr.*, **1988**, *432*, 308-314

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Serum + 10 mL dichloromethane, shake for 1 h, centrifuge for 15 min. Discard the aqueous layer, wash the organic layer with 1 mL 100 mM NaOH and 1 mL water (vortex for 30 s and centrifuge for 15 min each time). Remove the organic layer and evaporate it to dryness under a stream of nitrogen at $40-45^{\circ}$, reconstitute the residue in 200 μ L mobile phase, inject a 50 μ L aliquot onto column A in series with column B and elute with mobile phase. After 2.1 min remove column A from the circuit and backflush it with mobile phase at 1 mL/min for 15 min, elute column B with mobile phase and monitor the effluent.

HPLC VARIABLES**Column:** A 30 mm long Spherisorb silica; B 250 \times 4.6 6 μ m Zorbax silica**Mobile phase:** Butyl chloride:THF:MeOH:phosphoric acid 880:100:15:0.5 (Butyl chloride was 50% water-saturated.)**Flow rate:** 2**Injection volume:** 50**Detector:** UV 236

CHROMATOGRAM**Retention time:** 24**Internal standard:** 6 α -methylprednisolone

OTHER SUBSTANCES

Extracted: fluoxymesterone

KEY WORDS

serum; normal phase; column-switching; 6 α -methylprednisolone is IS

REFERENCE

Capponi, V.J.; Cox, S.R.; Harrington, E.L.; Wright, C.E.; Antal, E.J.; Albert, K.S. Liquid chromatographic assay for fluoxymesterone in human serum with application to a preliminary bioavailability study. *J.Pharm.Sci.*, **1985**, *74*, 308-311

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 80 μ L 3.125 μ g/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 μ L mobile phase, inject.

HPLC VARIABLES

Guard column: 70 \times 6 37-53 μ m Whatman HC-Pellocil

Column: 250 \times 4.6 5-6 μ m 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: 12

Internal standard: dexamethasone (8)

Limit of detection: 2 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: beclomethasone, betamethasone, corticosterone, cortisone, fluocinonide, hydrocortisone, methylprednisone, prednisolone, prednisone

KEY WORDS

plasma; normal phase

REFERENCE

Ebling, W.F.; Szeffler, 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: 1 mL Plasma + 100 μ L 2 μ g/mL dexamethasone in EtOH:water 10:90 + 100 μ L 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 μ L dichloromethane:EtOH:water 95:4:1, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 5 μ m Partisil silica

Mobile phase: Dichloromethane:EtOH:water 95:4:1

Flow rate: 1.5

Injection volume: 50

Detector: UV 239

CHROMATOGRAM

Retention time: 18

Internal standard: dexamethasone (11.5)

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 17-hydroxyprogesterone, prednisolone, prednisone, progesterone

KEY WORDS

plasma; normal phase

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: formulations

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb CN

Mobile phase: MeCN:100 mM pH 4.5 NaH₂PO₄ 15:85

Injection volume: 20

Detector: UV 300

CHROMATOGRAM

Retention time: 4.2

Internal standard: propylparaben (3.3)

OTHER SUBSTANCES

Simultaneous: dexamethasone, granisetron

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: Prepare a solution in chloroform:n-butanol 95:5, inject a 5 μ L aliquot.

HPLC VARIABLES

Guard column: 5 μ m Guard-Pak Resolve Si (dead volume 60-75 μ L)

Column: 75 \times 3.9 4 μ m Nova-Pak silica

Mobile phase: Dichloromethane:EtOH 34:1

Flow rate: 0.7
Injection volume: 5
Detector: UV 240

CHROMATOGRAM

Retention time: 10.5 (6 α -methylprednisolone)

OTHER SUBSTANCES

Simultaneous: betamethasone, cortisone, dexamethasone, hydrocortisone, prednisolone
Interfering: prednisone

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 betamethasone and dexamethasone. *J.Chromatogr.A*, **1994**, 676, 455–460

SAMPLE

Matrix: formulations
Sample preparation: Dilute 20-fold with mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18
Mobile phase: MeCN:50 mM pH 5.7 sodium acetate 33:67
Flow rate: 1
Injection volume: 10
Detector: UV 248

CHROMATOGRAM

Retention time: 6.4 (methylprednisolone sodium succinate)

KEY WORDS

water; injections; stability-indicating

REFERENCE

Nahata, M.C.; Morosco, R.S.; Hipple, T.F. Stability of diluted methylprednisolone sodium succinate injection at two temperatures. *Am.J.Hosp.Pharm.*, **1994**, 51, 2157–2159

SAMPLE

Matrix: perfusate
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. Add 3 mL Perfusate 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 $^{\circ}$, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 Newguard RP-18
Column: two 250 \times 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:** 30**Internal standard:** 6 α -methylprednisolone

OTHER SUBSTANCES**Extracted:** metabolites, cortisone, dihydrocortisol, dihydrocortisone, hydrocortisone**Simultaneous:** prednisolone**Noninterfering:** acetaminophen, albuterol, betamethasone, bupivacaine, carbamazepine, cholesterol, clonazepam, dehydroepiandrosterone, dexamethasone, diazepam, estradiol, estriol, hydroxyprogesterone, methimazole, phenobarbital, prednisone, progesterone, ritodrine, scopolamine, testosterone

KEY WORDSSPE; methylprednisolone is IS

REFERENCEDodds, 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:** Inject an aliquot of a 1 μ M solution in MeOH.

HPLC VARIABLES**Column:** 470 \times 4.6 5 μ m Spheri-5 RP-18**Mobile phase:** MeOH:water 56:44**Flow rate:** 0.5**Injection volume:** 10**Detector:** UV 240

CHROMATOGRAM**Retention time:** 55

OTHER SUBSTANCES**Simultaneous:** cortisone, dehydrocorticosterone, hydrocortisone, prednisolone, prednisone, tetrahydrocortisol, tetrahydrocortisone

REFERENCELukulay, 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

HPLC VARIABLES**Column:** 250 \times 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, benzotrope, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazeoxide, 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, dipyron, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, 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, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methyltestosterone, methylprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, pyrillamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, 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' 1.509 (methylprednisolone), k' 2.190 (methylprednisolone acetate)

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (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), 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 20 μ L aliquot of a MeOH solution.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Hypersil 5-ODS

Mobile phase: THF:water 23:77

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 245

CHROMATOGRAM

Retention time: k' 11.36

OTHER SUBSTANCES

Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fludrocortisone, fludrocortisone acetate, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, 11 α -hydroxyprogesterone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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

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: synovial fluid

Sample preparation: 100 μL Synovial fluid + 10 μL 10 $\mu\text{g}/\text{mL}$ flumethasone in MeOH + 1 mL 100 mM NaOH + 10 mL dichloromethane, shake for 10 min, centrifuge at 8400 g for 10 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, vortex, inject the whole amount.

HPLC VARIABLES

Column: 100 \times 8 10 μm Radial Pak B silica (Waters)

Mobile phase: Dichloromethane:MeOH:glacial acetic acid 96.8:2.4:0.8

Flow rate: 1.4

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 8 (methylprednisolone), 4 (methylprednisolone acetate)

Internal standard: flumethasone (6)

Limit of detection: 200 ng/mL

KEY WORDS

normal phase; cow; pharmacokinetics

REFERENCE

Alvinerie, M.; Toutain, P.L. Determination of methylprednisolone and methylprednisolone acetate in synovial fluid using high-performance liquid chromatography. *J.Chromatogr.*, **1984**, *309*, 385–390

SAMPLE

Matrix: tissue

Sample preparation: Homogenize rat brain in 10 mL n-hexane:isopropanol 60:40 in a glass homogenizing tube with a PTFE pestle at 4° using 15 passes, transfer to an acid-washed glass tube, wash out homogenizing tube with three 2.5 mL aliquots of n-hexane:isopropanol 60:40, centrifuge with a table-top centrifuge, remove the supernatant, re-extract the pellet with 8 mL n-hexane:isopropanol 60:40. Combine the supernatants and filter (0.2 μm) them, evaporate to dryness under a stream of nitrogen, reconstitute the residue in n-hexane:isopropanol 60:40, inject an aliquot into the gradient elution system. Collect the methylprednisolone peak and rechromatograph isocratically.

HPLC VARIABLES

Column: 250 \times 4.6 5-6 μm Zorbax silica

Mobile phase: Gradient. A was n-hexane:isopropanol 60:40. B was n-hexane:isopropanol:water 56.7:37.8:5.5. A:B from 55:45 to 24:76 over 7 min, to 0:100 over 5 min, maintain at 0:100 for 22 min. (Methylprednisolone peak can be collected and rechromatographed isocratically at A:B 90:10 at 0.8 mL/min, retention time 7.17 min.)

Column temperature: 34

Flow rate: 1.8

Detector: UV 254

CHROMATOGRAM**Retention time:** 3.27**Limit of detection:** 4.9 ng**Limit of quantitation:** 9.8 ng

KEY WORDS

rat; brain; normal phase

REFERENCE

Murphy, E.J.; Slivka, A.P.; Rosenberger, T.A.; Horrocks, L.A. High-performance liquid chromatography separation and quantitation of methylprednisolone from rat brain. *Anal.Biochem.*, **1993**, 209, 339–342

SAMPLE**Matrix:** urine

Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na₂HPO₄, 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:** 14**Internal standard:** methylprednisolone

OTHER SUBSTANCES**Extracted:** cortisone, hydrocortisone**Simultaneous:** fluorocortisone

Noninterfering: corticosterone, deflazacort, deoxycorticosterone, fluorocortisone acetate, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, prednisolone, prednisone, triamcinolone acetate

KEY WORDS

methylprednisolone is IS

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: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na₂HPO₄, 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: 9
Internal standard: prednisone (6.3)

OTHER SUBSTANCES

Simultaneous: betamethasone, corticosterone, cortisone, dexamethasone, fluorocortisone, fluorocortisone acetate, hydrocortisone, hydroxyprogesterone, prednisolone, triamcinolone, triamcinolone acetonide

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: 1 mL Urine + 1 mL 1.5 M HCl + 500 µL water + 10 mL dichloromethane, shake 20 min, centrifuge at 250 g for 3 min. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute with 200 µL MeOH, inject 10-30 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Partisil 10 ODS-3
Mobile phase: Gradient. A was MeCN:water 28:72 containing 0.05% phosphoric acid and 0.05% acetone. B was MeCN:50 mM KH₂PO₄ 50:50. A:B 100:0 for 8 min then to 0:100 over 6 min
Flow rate: 2
Injection volume: 10-30
Detector: UV 220

CHROMATOGRAM

Retention time: 13.6
Internal standard: methylprednisolone

OTHER SUBSTANCES

Extracted: ibuprofen, ibuprofen metabolites

KEY WORDS

methylprednisolone is IS

REFERENCE

Lockwood, G.F.; Wagner, J.G. High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids. *J.Chromatogr.*, **1982**, *232*, 335–343

ANNOTATED BIBLIOGRAPHY

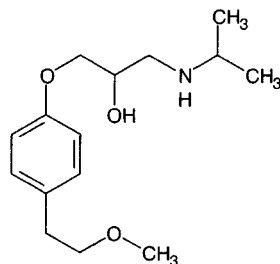
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- Valvo, L.; Paris, A.; Savella, A.L.; Gallinella, B.; Ciranni Signoretti, E. General high-performance liquid chromatographic procedures for the rapid screening of natural and synthetic corticosteroids. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 805–810 [gradient; reverse phase; normal phase; for 6 α -methylprednisolone, 6 α -methylprednisolone 21-acetate, 6 α -methylprednisolone 21-sodium succinate; also beclomethasone, beclomethasone 17,21-dipropionate, betamethasone, betamethasone 21-acetate, betamethasone 17,21-dipropionate, betamethasone 21-disodium phosphate, betamethasone 17-valerate, cortisone, cortisone 21-acetate, 11-deoxycorticosterone 21-acetate, dexamethasone, dexamethasone 21-acetate, dexamethasone 21-disodium phosphate, fluocinolone, fluocinolone acetonide, 9 α -fluorohydrocortisone 21-acetate, 9 α -fluorohydrocortisone, 9 α -fluoroprednisolone, 9 α -fluoroprednisolone 21-acetate, hydrocortisone, hydrocortisone 21-acetate, hydrocortisone 21-hemisuccinate, prednisolone, prednisolone 21-acetate, prednisolone 21-disodium phosphate, prednisolone 21-pivalate, prednisolone 21-sodium succinate, prednisone, triamcinolone, triamcinolone acetonide]
- Santos-Montes, A.; Gasco-Lopez, A.I.; Izquierdo-Hornillos, R. Optimization of the high-performance liquid chromatographic separation of a mixture of natural and synthetic corticosteroids. *J.Chromatogr.*, **1993**, *620*, 15–23 [simultaneous betamethasone, corticosterone, cortisone, deoxycorticosterone, dexamethasone, fluorocortisone, hydrocortisone, hydroxyprogesterone, prednisolone, prednisone, triamcinolone]
- McGinley, P.A.; Braugher, J.M.; Hall, E.D. Determination of methylprednisolone in central nervous tissue and plasma using normal-phase high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *230*, 29–35

Metoprolol

Molecular formula: C₁₅H₂₅NO₃

Molecular weight: 267.4

CAS Registry No.: 37350-58-6, 56392-17-7 (tartrate), 119637-66-0 (fumarate), 98418-47-4 (succinate)



SAMPLE

Matrix: blood

Sample preparation: Condition a Styrosorb cross-linked polystyrene (Biochrom, Moscow) or Sep-Pak C18 SPE cartridge with two 3 mL portions of MeOH and two 3 mL portions of water. Add 1 mL serum to the SPE cartridge, wash with two 3 mL portions of water, elute with 600 μ L MeOH:diethylamine 99.7:0.3. Evaporate the eluate to dryness under a stream of air at 40°, reconstitute with 50 μ L n-heptane:isopropanol:MeOH 83:13:4, inject a 20 μ L aliquot onto a 250 \times 4.6 10 μ m Silasorb-NH₂ (Elsico, Moscow) column and elute with n-heptane:isopropanol:MeOH 83:13:4 at 2.5 mL/min, collect the eluate containing metoprolol at about 4.1 min, evaporate to dryness, reconstitute with 30 μ L mobile phase, inject a 10-20 μ L aliquot.

HPLC VARIABLES

Guard column: 50 \times 4.6 10 μ m Chiralcel OD

Column: 250 \times 4.6 10 μ m Chiralcel OD

Mobile phase: n-Heptane:isopropanol:MeOH 77:8:15

Flow rate: 1.3

Injection volume: 10-20

Detector: F ex 220 em 320 (cut-off filter)

CHROMATOGRAM

Retention time: 3.5 (R), 4.6 (S)

KEY WORDS

SPE; serum; silanize glassware; chiral

REFERENCE

Rumiantsev, D.O.; Ivanova, T.V. Solid-phase extraction of Styrosorb cartridges as a sample pretreatment method in the stereoselective analysis of propranolol in human serum. *J.Chromatogr.B*, **1995**, *674*, 301-305

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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:** 4.20**Limit of detection:** <120 ng/mL**OTHER SUBSTANCES**

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, albuterol, alimemazine, alminoprofen, alpidem, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoyllecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cyproheptadine, cytarabine, dacarbazine, daunorubicin, demexiptiline, desipramine, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mepredine, mephenesin, mepivacaine, metapramine, metformin, methadone, methocarbamol, methotrexate, metipranolol, metoclopramide, mexiletine, mianserine, midazolam, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, niflumic acid, nimodipine, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxprenolol, penbutolol, penfluridol, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, prazepam, prazosin, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfinyprazole, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thio-properazine, thioridazine, tianeptine, tiapride, ticlopidine, timolol, tiocloamarol, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, zolpidem, zorubicine

Interfering: ajmaline, alprazolam, celiprolol, clobazam, cycloguanil, debrisoquine, dextromethorphan, disopyramide, ketamine, lorazepam, mephentermine, methaqualone, metoprolol, minoxidil, nifedipine, nitrazepam, oxazepam, pentazocine, piroxicam, prilocaine, quinidine, quinine, sulindac, tiaprofenic acid, tofisopam, yohimbine, zopiclone

KEY WORDS

whole blood; plasma

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 + 1 mL 5 µg/mL R-propranolol in water + 50 µL 100 mM NaOH + 4 mL chloroform, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove

organic layer and evaporate it to dryness in vacuum, reconstitute in 200 μL 0.05% S-(+)-1-(1-naphthyl)ethyl isocyanate in chloroform, vortex for 30 s, inject a 75-150 μL aliquot.

HPLC VARIABLES

Column: 250 mm long Whatman 5 μm silica
Mobile phase: Hexane:chloroform:MeOH 85:14:1
Flow rate: 2
Injection volume: 75-150
Detector: F ex 220 no emission filter

CHROMATOGRAM

Retention time: 14.1 (R-(+)), 16.2 (S-(-))
Internal standard: R-propranolol (8.5)
Limit of quantitation: 5 ng/mL

KEY WORDS

plasma; normal phase; derivatization; chiral

REFERENCE

Bhatti, M.M.; Foster, R.T. Stereospecific high-performance liquid chromatographic assay of metoprolol. *J.Chromatogr.*, **1992**, 579, 361-365

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 2 M NaOH + 4 mL dichloromethane, vortex for 10 s, centrifuge at 3000 rpm 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 an 80 μL aliquot.

HPLC VARIABLES

Guard column: NewGuard C18 (Brownlee)
Column: 250 \times 4.6 5 μm Dynamax Microsorb C18
Mobile phase: MeCN:0.1% triethylamine in water adjusted to pH 3.5 with 85% phosphoric acid 20:80
Flow rate: 1
Injection volume: 80
Detector: F ex 215

CHROMATOGRAM

Retention time: 11.65
Internal standard: metoprolol

OTHER SUBSTANCES

Simultaneous: pindolol

KEY WORDS

serum; metoprolol is IS

REFERENCE

Chmielowiec, D.; Schuster, D.; Gengo, F. Determination of pindolol in human serum by HPLC. *J.Chromatogr.Sci.*, **1991**, 29, 37-39

SAMPLE

Matrix: blood

Sample preparation: Condition an Analytichem 3 mL 200 mg SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 30 μ L 0.5 μ g/mL d-propranolol in MeOH, vortex for 15 s, add to the SPE cartridge, wash with 3 mL water, wash with 1 mL MeOH:water 50:50, elute with two aliquots of 500 μ L MeOH containing 0.1% triethylamine. Evaporate the eluate to dryness under nitrogen at 30°, reconstitute with 150 μ L mobile phase, vortex for 30 s, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: Chiracel OD (Daicel Chemical Industries)
Column: 250 \times 4.6 Chiracel OD (Daicel Chemical Industries)
Mobile phase: Hexane:EtOH:N,N-diethylamine 95:5:0.1
Flow rate: 0.5
Injection volume: 100
Detector: F ex 220 em 320

CHROMATOGRAM

Retention time: 19 (d), 22 (l)
Internal standard: d-propranolol (34)
Limit of detection: 4 ng/mL

KEY WORDS

plasma; SPE; chiral

REFERENCE

Herring, V.L.; Bastian, T.L.; Lalonde, R.L. Solid-phase extraction and direct high-performance liquid chromatographic determination of metoprolol enantiomers in plasma. *J.Chromatogr.*, **1991**, *567*, 221–227

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 2 mL MeCN and 1 mL MeCN:water 40:60. 995 μ L Serum + 5 μ L 20 μ M oxprenolol in water, add 20 μ L of this serum to 500 μ L MeCN:water 40:60, vortex, add to SPE cartridge, wash with 2 mL MeCN:water 40:60, elute with 1 mL MeOH:water 1:1 containing 0.05% trifluoroacetic acid. Evaporate eluate to dryness, reconstitute in MeCN:water 20:80 containing 0.05% trifluoroacetic acid, inject.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m TSK gel ODS 80Tm (Tosoh)
Mobile phase: MeCN:water 31:69 containing 0.05% trifluoroacetic acid
Column temperature: 40
Injection volume: 20
Detector: F ex 230 em 300

CHROMATOGRAM

Internal standard: oxprenolol

KEY WORDS

serum; SPE

REFERENCE

Uzu, S.; Imai, K.; Nakashima, K.; Akiyama, S. Use of 4-(N,N-dimethylaminosulphonyl)-7-fluoro-2,1,3-benzoxadiazole as a labelling reagent for peroxyoxalate chemiluminescence detection and its application to the determination of the β -blocker metoprolol in serum by high-performance liquid chromatography. *Analyst*, **1991**, *116*, 1353–1357

SAMPLE**Matrix:** blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 2 mL MeCN and 1 mL MeCN:water 40:60. Add 20 μ L serum to 500 μ L MeCN:water 40:60, vortex, add to SPE cartridge, wash with 2 mL MeCN:water 40:60, elute with 1 mL MeOH:water 1:1 containing 0.05% trifluoroacetic acid. Evaporate eluate to dryness under reduced pressure at 40°, reconstitute with 50 μ L 100 mM pH 9.0 borate buffer containing 2 mM EDTA, add 50 μ L 50 mM DBD-F in MeCN, heat at 45° for 8 h, add 100 μ L 100 mM acetic acid in MeCN:water 50:50, inject a 20 μ L 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 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 \times 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 \times 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 60F₂₅₄ 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.)

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m TSK gel ODS 80Tm (Tosoh)**Mobile phase:** MeCN:THF:50 mM pH 6.0 imidazole nitrate buffer 28:20:52**Column temperature:** 40**Flow rate:** 0.8**Injection volume:** 20**Detector:** Chemiluminescence following post-column reaction. The column effluent mixed with the reagent pumped at 1.4 mL/min and the mixture flowed to the detector. (Reagent was 0.25 mM bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate (TDPO, Wako, Osaka) and 37.5 mM hydrogen peroxide in MeCN:ethyl acetate 50:50.)**CHROMATOGRAM****Retention time:** 9**Limit of detection:** 0.8 ng/mL

KEY WORDS

SPE; derivatization; post-column reaction; serum

REFERENCE

Uzu, S.; Imai, K.; Nakashima, K.; Akiyama, S. Use of 4-(*N,N*-dimethylaminosulphonyl)-7-fluoro-2,1,3-benzoxadiazole as a labelling reagent for peroxyoxalate chemiluminescence detection and its application to the determination of the β -blocker metoprolol in serum by high-performance liquid chromatography. *Analyst*, **1991**, *116*, 1353–1357

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Serum + 50 μ L 1 μ g/mL verapamil in MeOH + 500 μ L 0.5 M HCl + 4 mL diethyl ether, vortex for 30 s, centrifuge at 2000 g for 5 min, discard organic layer, add 100 μ L 2 M NaOH and 4 mL diethyl ether to the aqueous layer, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the organic layer and dry it at 35° under a stream of helium. Reconstitute in 150 μ L mobile phase, inject a 25 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Chiralcel OD (Diacel Chemical Industries)**Mobile phase:** Hexane:isopropanol 90:10 which contained 10 mM octylamine**Flow rate:** 1**Injection volume:** 25**Detector:** F ex 275 em 315**CHROMATOGRAM****Retention time:** 6.13 (R), 14.34 (S)**Internal standard:** verapamil (9.80)**Limit of detection:** 5 ng/mL**OTHER SUBSTANCES****Simultaneous:** procainamide**Noninterfering:** diazepam, digoxin, furosemide, lidocaine, prochlorperazine, quinidine**KEY WORDS**

serum; chiral

REFERENCE

Straka, R.J.; Johnson, K.A.; Marshall, P.S.; Remmel, R.P. Analysis of metoprolol enantiomers in human serum by liquid chromatography on a cellulose-based chiral stationary phase. *J.Chromatogr.*, **1990**, *530*, 83–93

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Plasma + 1 mL 1 M pH 9.9 carbonate buffer + 6 mL water-saturated diethyl ether, agitate for 15 min, centrifuge at 4200 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 250 μ L 142.4 mM triethylamine in dichloromethane, add 100 μ L reagent, let stand for 15 min, evaporate to dryness under a stream of nitrogen at 35°, add 2 mL 100 mM NaOH, agitate for 10 min, add 6 mL ether, extract for 15 min, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, cool in an ice bath, reconstitute the residue in 250 μ L trifluoroacetic acid, let stand at 0° for 10 min, add 2 mL 2 M NaOH, extract with 6 mL ether. Remove a 5 mL aliquot of the organic layer and extract it with 100 μ L 100 mM phosphoric acid, inject a 94 μ L aliquot of the aqueous layer. (Purify triethylamine by drying it over NaOH pellets overnight, filter, add a volume of naphthylisocyanate equal to 2% of the volume of triethylamine, distill. Prepare reagent by dissolving 1 mmole *N*-tert-butoxycarbonyl-L-leucine (BOC-

L-Leu) in 3 mL dichloromethane, add 2 mL 250 mM dicyclohexylcarbodiimide in dichloromethane, let stand at 0° for 1 h, filter.)

HPLC VARIABLES

Column: 100 × 3.2 10 μm μBondapak C18

Mobile phase: MeCN:100 mM pH 3.0 phosphate buffer 30:70

Flow rate: 0.5

Injection volume: 94

Detector: F ex 193 em (no cutoff filter)

CHROMATOGRAM

Retention time: 5 (S), 6.5 (R)

Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Also analyzed: alprenolol

KEY WORDS

pharmacokinetics; plasma; chiral; derivatization

REFERENCE

Hermansson, J.; von Bahr, C. Determination of (R)- and (S)-alprenolol and (R)- and (S)-metoprolol as their diastereomeric derivatives in human plasma by reversed-phase liquid chromatography. *J.Chromatogr.*, **1982**, *227*, 113–127

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1-2 mL Plasma + 100 μL 4 M NaOH + 100 μL water + 5 mL dichloromethane, shake by hand for 10 s, vortex vigorously for 3 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, filter (0.45 μm), inject a 40-60 μL aliquot. Urine. 0.1-1 mL Urine + 100 μL 4 M NaOH + 100 μL water + 5 mL dichloromethane, vortex vigorously for 1 min, centrifuge at 1500 g for 5 min. Remove the organic layer and add it to 1 mL 100 mM phosphoric acid, vortex for 1 min, centrifuge at 1500 g for 5 min, inject a 40 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 Wakosil 5C18 (Wako)

Mobile phase: MeCN:water:triethylamine 18:81:1, pH adjusted to 3.0 with phosphoric acid

Flow rate: 1

Injection volume: 40-60

Detector: UV 295

CHROMATOGRAM

Retention time: 8.69

Internal standard: metoprolol tartrate

OTHER SUBSTANCES

Simultaneous: acebutolol, N-acetylprocainamide, alprenolol, atenolol, bufetolol, bupranolol, carteolol, disopyramide, indenolol, lidocaine, nifedipine, pindolol, procainamide, propranolol, quinidine, timolol

Noninterfering: diltiazem, glycinyxylidide, mexiletine, nicardipine, tocainide, verapamil

Interfering: nicainoprol

KEY WORDS

plasma; metoprolol is IS

REFERENCE

Kubota, K.; Nakamura, H.; Koyama, E.; Yamada, T.; Kikuchi, K.; Ishizaki, T. Simple and sensitive determination of timolol in human plasma and urine by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1990**, *533*, 255–263

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μ L Plasma or 100-200 μ L urine + 100 (plasma) or 150 (urine) μ L 10 (plasma) or 600 (urine) μ g/mL pindolol in MeOH + 0.5 mL 1 M NaOH + 3 mL dichloromethane, vortex for 1 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 35°, reconstitute the residue in 100 (plasma) or 200 (urine) μ L mobile phase, inject a 20-30 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax ODS

Mobile phase: MeCN:water:triethylamine 25:74:1, adjusted to pH 4.0 with phosphoric acid

Flow rate: 0.8

Injection volume: 20-30

Detector: F ex 230 em 300

CHROMATOGRAM

Retention time: 8.6

Internal standard: pindolol (6.0)

Limit of detection: 2 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Horai, Y.; Ishizaki, T.; Kusaka, M.; Tsujimoto, G.; Hashimoto, K. Simultaneous determination of metoprolol and α -hydroxymetoprolol in human plasma and urine by liquid chromatography with a preliminary observation on metoprolol oxidation in Japanese subjects. *Ther.Drug Monit.*, **1988**, *10*, 428–433

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. Add MeCN to blood so that the ratio is 1:5. Remove a 1 mL aliquot and add 1 mL water, adjust pH to 9.8-10.2 with 7-10 drops 100 mM NaOH, add 2 mL benzene (Caution! Benzene is a carcinogen!), shake on an automatic shaker for 30 min, centrifuge, remove the organic phase and extract the aqueous layer again with 2 mL benzene for 20 min. Combine the organic layers and evaporate them under reduced pressure at 30°, dissolve the residue in 50 μ L mobile phase, inject a 10-50 μ L aliquot. Urine. 20-1000 μ L Urine + 1 mL 200 mM pH 10.2 sodium borate buffer (Sørensen), adjust pH to 9.8-10.2 with 100 mM NaOH (if necessary), add 500 mg NaCl, extract with 4 mL benzene for 30 min, centrifuge. Remove the organic layer and evaporate it under reduced pressure at 30°, dissolve the residue in 50 μ L mobile phase, inject a 10-50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m μ Bondapak C18

Mobile phase: MeOH:water 48:52 containing 0.4% phosphoric acid and 0.2% heptanesulfonic acid

Flow rate: 2

Injection volume: 10-50
Detector: F ex 225 em 295

CHROMATOGRAM

Retention time: 4.6
Internal standard: metoprolol

OTHER SUBSTANCES

Simultaneous: dihydrolevobunolol

KEY WORDS

human; dog; metoprolol is IS

REFERENCE

Hengy, H.; Kölle, E.-U. Determination of levobunolol and dihydrolevobunolol in blood and urine by high-performance liquid chromatography using fluorescence detection. *J.Chromatogr.*, **1985**, 338, 444-449

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 10 μ mole compound (as free base or hydrochloride) in 500 μ L MeCN, add 250 μ L 5% sodium carbonate (for hydrochlorides only), add 500 μ L 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μ mole L-proline, heat at 60° for 30 min. Remove a 100 μ L aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μ L 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 μ L 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°, $[\alpha]_{D}^{25} = -133^\circ$ (c=1) in MeCN).)

HPLC VARIABLES

Column: 125 \times 4.5 μ m 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' 5.33, k' 7.83 (enantiomers)

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, 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: bulk

Sample preparation: Prepare a 2 mg/mL solution, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.5 μ m LiChrospher RP-select B C8

Mobile phase: MeCN:buffer 17:83 (Buffer was 11.5 g ammonium dihydrogen phosphate and 10 mL 1 M phosphoric acid made up to 2 L, pH 3.2.)

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Erickson, M.; Karlsson, K.-E.; Lamm, B.; Larsson, S.; Svensson, L.A.; Vessman, J. Identification of a new by-product detected in metoprolol tartrate. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 567–574

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 2 mg tablet or capsule in 10 mL pH 10 solution, extract twice with 2 mL ether, combine extracts, filter, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 μ m β -cyclodextrin bonded C18 (Advanced Separation Technologies)

Mobile phase: MeCN:MeOH:acetic acid:triethylamine 95:5:0.3:0.2

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 12, 13 (enantiomers)

OTHER SUBSTANCES

Simultaneous: atenolol, propranolol

KEY WORDS

capsules; tablets; chiral

REFERENCE

Tran, C.D.; Dotlich, M. Enantiomeric separation of beta-blockers by high performance liquid chromatography. *J.Chem.Educ.*, **1995**, *72*, 71–73

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: 10.5

OTHER SUBSTANCES

Simultaneous: atenolol, nadolol, alprenolol, acebutolol, oxprenolol, pindolol, practolol, propranolol, sotalol, timolol

KEY WORDS

tablets

REFERENCE

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

SAMPLE

Matrix: saliva

Sample preparation: Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. 1 mL Supernatant + 50 μL 10 μg/mL alprenolol, add to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

HPLC VARIABLES

Guard column: RCSS silica guard-pack (Waters)

Column: 250 × 4.6 Chiralcel OD-H

Mobile phase: n-Hexane:EtOH:diethylamine 91:8:1

Flow rate: 1

Injection volume: 40

Detector: F ex 225 em 320 cut-off filter

CHROMATOGRAM

Internal standard: (S)-alprenolol

KEY WORDS

SPE; chiral

REFERENCE

Höld, K.M.; de Boer, D.; Zuidema, J.; Maes, R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva. *J.Chromatogr.B*, **1995**, *663*, 103–110

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 ace-

tone, 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 \times 4.5 μm Zorbax-SIL

Mobile phase: n-Hexane:isopropanol 100:1.5

Flow rate: 1.5

Injection volume: 100

Detector: UV 230; F ex 270 em 365

CHROMATOGRAM

Retention time: k' 12.6 (S-(-)), k' 13.5 (R-(+))

Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Simultaneous: flecainide, propafenone, tocainide

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: 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 55:45

Flow rate: 1

Injection volume: 100

Detector: UV 230; F ex 270 em 350

CHROMATOGRAM

Retention time: k' 20.4 (S-(-)), 25.4 (R-(+))

KEY WORDS

derivatization; chiral; F not much more sensitive than UV; α = 1.25

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 CSP-4 (Prepare as follows. Add a solution of 1.07 g L-valyl-L-valyl-L-valine isopropylester (Bunseki Kagaku 1979, 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 Li-Chrosorb 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:EtOH:trifluoroacetic acid 62.5:35:0.625:0.25

Detector: UV

CHROMATOGRAM

Retention time: k' 1.22 (first enantiomer)

KEY WORDS

chiral; α = 1.05

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

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 25:75 (Buffer was 60 mM KH₂PO₄ adjusted to pH 3.0 with phosphoric acid.)**Flow rate:** 0.6-1**Injection volume:** 10-100**Detector:** UV 270

OTHER SUBSTANCES**Also analyzed:** oxprenolol

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 Chirex 3022 (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 276

KEY WORDSchiral; $\alpha = 1.08$

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:isopropanol:diethylamine 80:20:0.1**Flow rate:** 0.5**Injection volume:** 20**Detector:** UV 275

CHROMATOGRAM**Retention time:** k' 0.69, 2.02 (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**Sample preparation:** Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES**Column:** 100 \times 4.7 μm Hypercarb (Shandon)**Mobile phase:** MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH**Column temperature:** 17**Injection volume:** 20**Detector:** UV 270

CHROMATOGRAM**Retention time:** k' 18 (first enantiomer)

KEY WORDSchiral; $\alpha = 1.09$

REFERENCEHuynh, N.-H.; Karlsson, A.; Pettersson, C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglycyl-L-proline as counter ion in methanol. *J.Chromatogr.A*, **1995**, 705, 275–287

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 \times 4.6 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' 2.72

OTHER SUBSTANCES**Also analyzed:** acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimeti-dine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxa-zosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, moxonidine, nadolol, na-phazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyr-amine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleennamine, triprolidine, tymazoline, UK-14,304

REFERENCEKaliszan, 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 \times 4.6 μm Supelcosil LC-DP (A) or 250 \times 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: 7.12 (A), 4.19 (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, hydroalazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methylodopa, methylphenidate, metoclopramide, metolazone, 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, sulfipyrazone, 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

some 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 × 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' 1.61

KEY WORDS

chiral; $\alpha = 1.24$

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: 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' 2.81

OTHER SUBSTANCES

Also analyzed: acebutolol, bunitrolol, carazolol, celiprolol, esmolol, mepindolol, 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: 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, benzotropane, benzphetamine, berberine, bibucaine, bromazepam, 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, dipyrrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, 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, isocarboxystiril, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, mibolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyliadin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperacaine, piperzepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrihydione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, 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 Chiralpak OD (Daicel)

Mobile phase: Carbon dioxide:MeOH:diethylamine 69.5:30:0.5

Column temperature: 30

Flow rate: 2

Detector: UV 223

CHROMATOGRAM

Retention time: 2, 3.1 (enantiomers)

KEY WORDS

SFC; chiral; pressure 200 bar

REFERENCE

Kot, A.; Sandra, P.; Venema, A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs. *J.Chromatogr.Sci.*, **1994**, *32*, 439-448

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 25 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: 7.53

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, diltiazem, 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

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 KH₂PO₄ 10:90

Flow rate: 1

Injection volume: 10

Detector: UV 232-274 (wavelength of maximum absorption used)

CHROMATOGRAM

Retention time: 26.0

OTHER SUBSTANCES

Simultaneous: acebutolol, alprenolol, atenolol, carteolol, oxprenolol, pindolol

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: Prepare a 10 $\mu\text{g/mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 \times 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, bamethane, 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, dipyrizamide, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mephivacaine, mepytazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, 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, pimozone, pindolol, pipazine, 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, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, 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: urine

Sample preparation: 2 mL Urine + 30 μ L 1 mg/mL (\pm)-toliprolol in MeOH + 1 mL 2 M potassium carbonate + 1 g NaCl, extract twice with 5 mL ethyl acetate. Remove the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L 0.4% triethylamine in MeCN:MeOH 50:50, add 100 μ L 1% S(-)-menthyl chloroformate in MeCN (prepare weekly in MeCN dried over anhydrous sodium sulfate), let stand at room temperature for 1 h, evaporate to dryness under a stream of nitrogen, reconstitute with 300 μ L MeOH, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4 30 μ m Hypersil HP ODS

Column: 250 \times 4.6 Hypersil 5 C18

Mobile phase: Gradient. A was 13.8 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 1.59 g propylamine hydrochloride in 1 L water, pH adjusted to 3.2 with concentrated phosphoric acid. B was MeOH. A:B from 25:75 to 15:85 over 15 min, maintain at 15:85 for 5 min, to 10:90 over 5 min, maintain at 10:90 for 3 min.

Flow rate: 1

Injection volume: 10

Detector: F ex 223 em 340 (cut-off filter)

CHROMATOGRAM

Retention time: 19.7 (-), 20.6 (+)

Internal standard: toliprolol (23.3 (-), 24.5 (+))

Limit of detection: 5 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

chiral; derivatization; pharmacokinetics

REFERENCE

Li, F.; Cooper, S.F.; Côté, M. Determination of the enantiomers of metoprolol and its major acidic metabolite in human urine by high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.B*, **1995**, *668*, 67–75

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 500 μ L 5 M NaOH + 1 g anhydrous sodium sulfate + 2 mL diethyl ether, shake mechanically for 15 min, centrifuge at 734 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:water 40:60 containing 5 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ adjusted to pH 6.5 with phosphoric acid or 1 M KOH

Column temperature: 30 \pm 0.2

Flow rate: 1.3

Injection volume: 20

Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon cell +1300 mV, d.c. mode, Ag/AgCl reference electrode (At the end of each day clean electrode with MeOH as mobile phase and potential -600 mV for 1 min and +1500 mV for 10 min, repeat 3 times. If necessary, wipe with a tissue wetted with water then a tissue wetted with MeOH.)

CHROMATOGRAM

Retention time: 5.42

Limit of quantitation: 400 ppb

OTHER SUBSTANCES

Extracted: alprenolol, nadolol, oxprenolol, timolol

Simultaneous: atenolol

REFERENCE

Maguregui, M.I.; Alonso, R.M.; Jiménez, R.M. High-performance liquid chromatography with amperometric detection applied to the screening of β -blockers in human urine. *J.Chromatogr.B*, **1995**, **674**, 85-91

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 \times 4.6 5 μ m Spherisorb cyanopropyl; B 250 \times 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 23:77 (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 (gradient), 6 (isocratic)

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

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

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

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Welch, C.J.; Perrin, S.R. Improved chiral stationary phase for β -blocker enantioseparations. *J.Chromatogr.A*, **1995**, *690*, 218–225 [chiral; also bufuralol]

Xie, H.G.; Zhou, H.H. Assay of metoprolol and alpha-hydroxymetoprolol in human urine by reversed-phase liquid chromatography with direct-injection. *Chung Kuo Yao Li Hsueh Pao*, **1995**, *16*, 32–35

Bailey, C.J.; Ruane, R.J.; Wilson, I.D. Packed-column supercritical fluid chromatography of β -blockers. *J.Chromatogr.Sci.*, **1994**, *23*, 426–429 [SFC; simultaneous alprenolol, atenolol, labetalol, metoprolol, oxprenolol, pindolol, practolol, propranolol, toliprolol, xamoterol]

Hermansson, J.; Grahn, A. Resolution of racemic drugs on a new chiral column based on silica-immobilized cellobiohydrolase. Characterization of the basic properties of the column. *J.Chromatogr.*, **1994**, *687*, 45–59 [chiral; also acebutolol, atenolol, betaxolol, bisoprolol, carbutoleol, cathinone, cimetidine, dobutamine, dopropizine, epanolol, epinephrine, laudanosine, metanephrine, moprolool, norepinephrine, normetanephrine, octopamine, oxybutynine, pamatolol, practolol, prilocaine, propafenone, proxphylline, sotalol, talinolol, tetrahydropapaveroline, tetramisole, timolol, tolamolol, toliprolol]

Armstrong, D.W.; Chen, S.; Chang, C.; Chang, S. A new approach for the direct resolution of racemic beta adrenergic blocking agents by HPLC. *J.Liq.Chromatogr.*, **1992**, *15*, 545–556 [chiral; also alprenolol, atenolol, cateolol, labetalol, nadolol, oxprenolol, pindolol, propranolol, timolol]

Leloux, M.S. Rapid chiral separation of metoprolol in plasma—application to the pharmacokinetics/pharmacodynamics of metoprolol enantiomers in the conscious goat. *Biomed.Chromatogr.*, **1992**, *6*, 99–105

Balmér, K.; Persson, A.; Lagerström, P.-O.; Persson, B.-A.; Schill, G. Liquid chromatographic separation of the enantiomers of metoprolol and its alpha-hydroxy metabolite on Chiralcel OD for determination in plasma and urine. *J.Chromatogr.*, **1991**, *553*, 391–397 [plasma; urine; human; dog; extracted metabolites; fluorescence detection; chiral; column temp 35; column temp 25; LOD 10 nM]

Shen, J.; Wanwimolruk, S.; Hung, C.T.; Zoest, A.R. Quantitative analysis of β -blockers in human plasma by reversed-phase ion-pair high-performance liquid chromatography using a microbore column. *J.Liq.Chromatogr.*, **1991**, *14*, 777–793 [plasma; microbore; also atenolol, labetalol, pindolol, propranolol; oxprenolol (IS); fluorescence detection; UV detection; LOD 1-10 ng/mL]

Persson, B.A.; Balmer, K.; Lagerstrom, P.O.; Schill, G. Enantioselective determination of metoprolol in plasma by liquid chromatography on a silica-bonded alpha 1-acid glycoprotein column. *J.Chromatogr.*, **1990**, *500*, 629–636

Bui, K.H.; French, S.B. Direct serum injection and analysis of drugs with aqueous mobile phases containing triethylammonium acetate. *J.Liq.Chromatogr.*, **1989**, *12*, 861–873 [direct injection; serum; plasma; dog; rat; fluorescence detection; UV detection; also antipyrine, atenolol, hexanophenone, naproxen, propranolol]

Rutledge, D.R.; Garrick, C. Rapid high-performance liquid chromatographic method for the measurement of the enantiomers of metoprolol in serum using a chiral stationary phase. *J.Chromatogr.*, **1989**, *497*, 181–190

Schuster, D.; Modi, M.W.; Lalka, D.; Gengo, F.M. Reversed-phase high-performance liquid chromatographic assay to quantitate diastereomeric derivatives of metoprolol enantiomers in plasma. *J.Chromatogr.*, **1988**, *433*, 318–325

Buhring, K.U.; Garbe, A. Determination of the new beta-blocker bisoprolol and of metoprolol, atenolol and propranolol in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *382*, 215–224

Schill, G.; Wainer, I.W.; Barkan, S.A. Chiral separation of cationic drugs on an α 1-acid glycoprotein bonded stationary phase. *J.Liq.Chromatogr.*, **1986**, *9*, 641–666 [chiral; also atropine, bromdiphen-

hydramine, brompheniramine, bupivacaine, butorphanol, carbinoxamine, chlorpheniramine, clidinium, cocaine, cyclopentolate, dimethindene, dipiperidone, disopyramide, doxylamine, ephedrine, homatropine, labetalol, labetalol A, labetalol B, mepensolate, mepivacaine, methadone, methorphan, methylatropine, methylhomatropine, methylphenidate, metoprolol, nadolol, nadolol A, nadolol B, oxprenolol, oxyphenyclimine, phenmetrazine, phenoxybenzamine, promethazine, pronethalol, propoxyphene, propranolol, pseudoephedrine, terbutaline, tocanide, tridihexethyl]

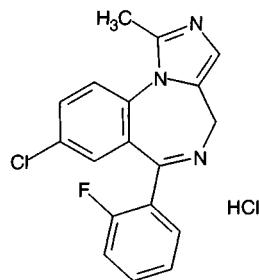
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- Winkler, H.; Ried, W.; Lemmer, B. High-performance liquid chromatographic method for the quantitative analysis of the aryloxypropanolamines propranolol, metoprolol and atenolol in plasma and tissue. *J.Chromatogr.*, **1982**, 228, 223–234
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Midazolam

Molecular formula: C₁₆H₁₃ClFN₃

Molecular weight: 325.8

CAS Registry No.: 59467-70-8 (midazolam), 59467-96-8 (midazolam hydrochloride), 59467-94-6 (midazolam maleate)



SAMPLE

Matrix: aqueous humor, blood, tissue, urine

Sample preparation: Homogenize tissue 1:2 (w/v). 1 mL Sample + 100 μ L 10 μ g/mL methaqualone + 1 mL ammonium chloride/ammonium hydroxide buffer (pH 9.2) + 3 mL n-butyl chloride, mix, centrifuge. Remove the organic layer and evaporate it to dryness under nitrogen at 45°, reconstitute the residue with 50 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 8 10 μ m μ Bondapak C18

Mobile phase: MeCN:buffer 40:60, pH 3.3 (Buffer was 150 mL 100 mM KH₂PO₄ made up to 1 L, pH adjusted to 3.3 with 100 mM phosphoric acid.)

Flow rate: 2.5

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 3.93

Internal standard: methaqualone (6.42)

KEY WORDS

plasma; liver; kidney

REFERENCE

Ferslew, K.E.; Hagardorn, A.N.; McCormick, W.F. Postmortem determination of the biological distribution of sufentanil and midazolam after an acute intoxication. *J. Forensic Sci.*, **1989**, *34*, 249–257

SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. 5 mL Plasma + 250 ng detomidine, add 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Hitachi gel

Mobile phase: 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 326

CHROMATOGRAM**Retention time:** 10.5**Internal standard:** detomidine (m/z 187) (6.5)**Limit of quantitation:** 1-2 ng/mL

OTHER SUBSTANCES**Extracted:** atipamazole, medetomidine

KEY WORDS

pig; plasma; pharmacokinetics; SPE

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:** blood**Sample preparation:** 1 mL Plasma + 300 μ L 100 mM pH 9 borate buffer + 25 μ L flurazepam in EtOH + 5 mL diethyl ether, mix at 60 rpm for 10 min, centrifuge at 15° at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Spherisorb CN**Mobile phase:** MeOH:isopropanol 75:25 containing 0.015% perchloric acid**Flow rate:** 1.5**Detector:** UV 215

CHROMATOGRAM**Retention time:** 4.7**Internal standard:** flurazepam (6.2)**Limit of quantitation:** 2 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDS

plasma

REFERENCE

Lehmann, B.; Boulieu, R. Determination of midazolam and its unconjugated 1-hydroxy metabolite in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *674*, 138-142

SAMPLE**Matrix:** blood**Sample preparation:** 600 μ L Plasma + 600 μ L IS solution, shake, centrifuge at 1500 g for 3 min, inject a 400 μ L aliquot onto column A with mobile phase A, elute with mobile phase A for 4 min, backflush column A with mobile phase A for 1.5 min, backflush column A with mobile phase B for 4.5 min, backflush contents of column A onto column B with mobile phase C and start the gradient. After 3 min remove column A from circuit, monitor effluent from column B. (IS solution was 2.5 mL 200 μ g/mL flurazepam in MeCN + 3.6 mL 2 M NaOH, add 200 mL MeCN, make up to 1 L with water.)

HPLC VARIABLES

Column: A 17 × 4.6 37-50 μm Bondapak C18 Corasil; B 4 × 4 5 μm LiChrospher 60 RP-select B + 250 × 4 5 μm LiChrospher 60 RP-select B

Mobile phase: A 100 mM NaOH; B 2.7 g/L KH₂PO₄ adjusted to pH 8.0 with 2 M NaOH; C Gradient. I was 2.7 g/L KH₂PO₄ adjusted to pH 2.4 with 85% phosphoric acid. II was MeCN. I:II from 76:24 to 66:34 over 11 min.

Flow rate: A 1; B 1; C 1.5

Injection volume: 400

Detector: UV 230

CHROMATOGRAM

Retention time: 20.5

Internal standard: flurazepam (21.5)

Limit of detection: 2 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Lauber, R.; Mosiman, M.; Bühner, M.; Zbinden, A.M. Automated determination of midazolam in human plasma by high-performance liquid chromatography using column switching. *J.Chromatogr.B*, **1994**, *654*, 69–75

SAMPLE

Matrix: blood

Sample preparation: Condition a 12 mL 500 mg PrepSep C1 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 100 μL 3 μg/mL midazolam in MeOH, mix, add to SPE cartridge, wash with two 3 mL portions of water, wash with two 1 mL portions of MeOH:water 30:70, elute with two 1 mL portions of MeOH:50 mM pH 9.0 (NH₄)₂HPO₄ 90:10, evaporate the eluents under vacuum, dissolve the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Spherisorb C8

Mobile phase: MeCN:MeOH:20 mM (NH₄)H₂PO₄ 5:35:60 containing 2 mL/L 200 mM tetrabutylammonium bromide, final pH adjusted to 4.10

Column temperature: 30

Flow rate: 1.5

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 10.2

Internal standard: clonazepam (12.4)

Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; SPE

REFERENCE

Mastey, V.; Panneton, A.-C.; Donati, F.; Varin, F. Determination of midazolam and two of its metabolites in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *655*, 305–310

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 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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: 7.6

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylcegonine, 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, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocinide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

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:** Condition a 100 mg Bond-Elut C2 SPE cartridge with 1 volume MeOH and 1 volume 10 mM pH 8.0 phosphate buffer. 1 mL Plasma + 5 µg prazepam + 100 µL 1 M pH 8.0 potassium phosphate buffer, mix, add to the SPE cartridge, wash with 3 volumes of water, wash with 1 mL MeOH:water 30:70, wash with 1 mL water, elute with 1 mL MeOH:water 70:30, elute with 1 mL water. Evaporate the eluate to dryness, reconstitute with 200 µL mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 35 × 4.6 5 µm Ultrabase C18**Mobile phase:** MeOH:water 60:40**Flow rate:** 1**Injection volume:** 20**Detector:** UV 217

CHROMATOGRAM**Retention time:** 4**Internal standard:** prazepam (8)**Limit of detection:** 3 ng/mL**Limit of quantitation:** 5 ng/mL

KEY WORDS

plasma; SPE

REFERENCEBerrueta, L.A.; Gallo, B.; Vincente, F. Rapid determination of midazolam in plasma using SPE and HPLC. *Am.Lab.*, 1993, 25 (Dec.), 20R-20T

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:** 12.7**Limit of detection:** 0.5-2.5 ng/mL

OTHER SUBSTANCES**Simultaneous:** atipamezole, atropine, butorphanol, flumazenil, ketamine, medetomidine, 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: blood

Sample preparation: Condition a 100 mg C18 Bond-Elut SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 100 μ L 10 μ g/mL climazolam in MeOH + 1 mL MeCN:water 30:70, vortex for 10 s, centrifuge for 5 min at 4000 g, add to the SPE cartridge, wash with 2 mL MeCN:water 15:85, let dry for 3-4 min, elute with four 200 μ L aliquots of MeOH. Evaporate the eluate under nitrogen, take up the residue in 100 μ L MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4.5 μ m LiChrosorb 100 RP 18

Column: 125 \times 4.5 μ m LiChrospher 100 RP 18 endcapped

Mobile phase: MeCN:MeOH:THF:buffer 28:25:2:50 (Prepare a 1 M pH 5.6 phosphate buffer from 94.8 mL 1 M KH_2PO_4 + 5.2 mL 1 M K_2HPO_4 . Dilute 10 mL of this buffer to 1 L to give the 10 mM pH 5.6 phosphate buffer used in the mobile phase.)

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 7.48

Internal standard: climazolam (9.79)

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; SPE

REFERENCE

Sautou, V.; Chopineau, J.; Terrisse, M.P.; Bastide, P. Solid-phase extraction of midazolam and two of its metabolites from plasma for high-performance liquid chromatographic analysis. *J.Chromatogr.*, **1991**, *571*, 298-304

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 25 μ L EtOH + 25 μ L 3 μ g/mL IS1 and 7.6 μ g/mL IS2 in EtOH + 1 mL 100 mM Na_2HPO_4 adjusted to pH 10.5 with NaOH + 5 mL diethyl ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 4° at 2000 g for 10 min. Remove the organic phase and add it to 1 mL 100 mM Na_2HPO_4 adjusted to pH 10.5 with NaOH, vortex for 30 s, centrifuge at 4° at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μ L mobile phase, inject a 1-15 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m CP-Microspher C18 (Chrompack)

Mobile phase: Gradient. A was MeOH:buffer 1:2. B was MeOH:water 80:20. A:B 93.8:6.2 for 5.5 min, to 60:40 over 0.15 min, maintain at 60:40 for 11.3 min, to 2.5:97.5 over 0.5 min, maintain at 2.5:97.5 for 3.5 min, return to initial conditions over 0.5 min (Buffer was 6 g/L NaH_2PO_4 and 1 mL/L triethylamine adjusted to pH 7.00 with NaOH.)

Column temperature: 40
Flow rate: 1.5
Injection volume: 1-15
Detector: UV 220

CHROMATOGRAM

Retention time: 15.5
Internal standard: IS1 ethyl 7-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (Ro 15-305) (6.4); IS2 clomazepam (17.0)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: flumazenil

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Vletter, A.A.; Burm, A.G.L.; Breimer, L.T.M.; Spierdijk, J. High-performance liquid chromatographic assay to determine midazolam and flumazenil simultaneously in human plasma. *J.Chromatogr.*, 1990, 530, 177-185

SAMPLE

Matrix: blood, CSF, gastric fluid, urine

Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m 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: 14.69

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazeponide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, oxazepam, para-oxon, 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: Condition 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 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 35 \times 4.6 5 μ m ultrabase C18 (Scharlau)

Mobile phase: MeOH:water 60:40

Flow rate: 1

Injection volume: 20

Detector: UV 217

CHROMATOGRAM

Retention time: 4

Internal standard: prazepam (8)

Limit of detection: 93 ng/mL

OTHER SUBSTANCES

Also analyzed: adinazolam, brotizolam, diazepam, nordazepam, oxazepam, temazepam

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: blood, urine

Sample preparation: 500 μ L Plasma or urine + 50 μ L 4 μ g/mL flurazepam in MeOH + 1 mL 100 mM pH 9 sodium phosphate buffer + 4 mL dichloromethane:diethyl ether 60:40, shake at 45 rpm for 15 min, centrifuge at 10° at 1870 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 80 μ L MeOH, inject a 30 μ L aliquot. (Deconjugate urine as follows. 250 μ L Urine + 750 μ L pH 5.4 acetate buffer + 500 U β -glucuronidase, heat at 37° for 18 h, add 20 μ L 5 M NaOH, centrifuge, proceed as above using 5 mL dichloromethane:diethyl ether.)

HPLC VARIABLES

Guard column: 30 \times 4.6 30 μ m C8

Column: 100 \times 8 4 μ m Nova Pak C18

Mobile phase: MeCN:40 mM sodium phosphate buffer 32:68 containing 1 mL/L triethylamine, final pH 7.2

Flow rate: 1.5

Injection volume: 30

Detector: UV 220

CHROMATOGRAM**Retention time:** 33.5**Internal standard:** flurazepam (18.5)**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** flumazenil, 1-hydroxymethylmidazolam, 4-hydroxymidazolam**Noninterfering:** alfentanil, atropine, bupivacaine, lignocaine, neostigmine

KEY WORDS

plasma

REFERENCE

Chan, K.; Jones, R.D.M. Simultaneous determination of flumazenil, midazolam and metabolites in human biological fluids by liquid chromatography. *J.Chromatogr.*, **1993**, *619*, 154–160

SAMPLE**Matrix:** formulations

HPLC VARIABLES**Column:** 300 × 3.9 μBondapak C18**Mobile phase:** MeCN:THF:dioxane:4.5 mM pH 7.0 potassium phosphate buffer 17.5:10:17.5:55 (Caution! Dioxane is a carcinogen!)**Flow rate:** 1**Detector:** UV 254

CHROMATOGRAM**Retention time:** 10.3

OTHER SUBSTANCES**Simultaneous:** benzyl alcohol

KEY WORDS

injections; saline; stability-indicating

REFERENCE

McMullin, S.T.; Burns Schaiff, R.A.; Dietzen, D.J. Stability of midazolam hydrochloride in polyvinyl chloride bags under fluorescent light. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2018–2020

SAMPLE**Matrix:** formulations**Sample preparation:** Inject an aliquot directly.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm C18-modified HS silica (Vydac)**Mobile phase:** MeOH:MeCN:THF:buffer 29.4:29.4:1.2:40 (Buffer was 6.1 mL K₂HPO₄ + 3.9 mL 1 M KH₂PO₄ made up to 1 L.)**Injection volume:** 10**Detector:** UV 220; UV 254

CHROMATOGRAM**Retention time:** 5.1

OTHER SUBSTANCES**Simultaneous:** degradation products, benzyl alcohol

KEY WORDS

injections; stability-indicating; saline; 5% dextrose

REFERENCE

Hagan, R.L.; Jacobs, L.F., III; Pimsler, M.; Merritt, G.J. Stability of midazolam hydrochloride in 5% dextrose injection or 0.9% sodium chloride injection over 30 days. *Am.J.Hosp.Pharm.*, **1993**, *50*, 2379-2381

SAMPLE

Matrix: formulations

Sample preparation: Dilute with saline, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:100 mM K₂HPO₄ 33:67 adjusted to a final pH of 4.4 with phosphoric acid

Flow rate: 1.7

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5.1

OTHER SUBSTANCES

Interfering: promethazine

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: 200 μ L Microsomal incubation + 200 μ L cold MeOH:MeCN 35:21 + flunitrazepam in MeOH, centrifuge at 10000 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: Zorbax RX-C18

Column: 250 \times 4.6 Zorbax RX-C18

Mobile phase: MeCN:MeOH:10 mM pH 7.4 potassium phosphate buffer 21:35:44

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 17.5

Internal standard: flunitrazepam (7.5)

KEY WORDS

human; liver

REFERENCE

Ring, B.J.; Binkley, S.N.; Roskos, L.; Wrighton, S.A. Effect of fluoxetine, norfluoxetine, sertraline and desmethyl sertraline on human CYP3A catalyzed 1'-hydroxy midazolam formation *in vitro*. *J.Pharmacol.Exp.Ther.*, **1995**, *275*, 1131-1135

SAMPLE

Matrix: microsomal incubations

Sample preparation: Add an equal volume of EtOH to the microsomal incubation, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 μm RP18 Lichrospher 100

Column: 150 × 4.6 3 μm Supelcosil LC-8

Mobile phase: MeCN:MeOH:100 mM pH 6 ammonium acetate 17:26:57

Column temperature: 40

Flow rate: 2

Detector: UV 254

KEY WORDS

monkey; mouse; rat; dog; human

REFERENCE

Valles, B.; Schiller, C.D.; Coassolo, P.; De Sousa, G.; Wyss, R.; Jaeck, D.; Viger-Chougnnet, A.; Rahmani, R. Metabolism of mofarotene in hepatocytes and liver microsomes from different species. Comparison with *in vivo* data and evaluation of the cytochrome P450 isoenzymes involved in human biotransformation. *Drug Metab.Dispos.*, **1995**, *23*, 1051-1057

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: 10.21 (A), 6.30 (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, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydro-

xyzine, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, 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, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 + 3 mL 500 mM NaOH, vortex for 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 under a stream of nitrogen, reconstitute the residue in 2 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 × 5 5 μm Waters Radial-Pak C18

Mobile phase: MeOH:200 mM NaCl 65:35

Flow rate: 1.2

Injection volume: 50

Detector: E, Bioanalytical Systems LC4B, glassy carbon working electrode operated in parallel mode, stainless steel auxiliary electrode, electrode potentials +1.0 V and 0.85 V, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 9.144 m × 0.5 mm i.d. figure eight coil of PTFE tubing in a UV irradiation unit maintained at 0–5° with an ice bath to the detector.

CHROMATOGRAM

Limit of detection: 22 ng/mL

OTHER SUBSTANCES

Also analyzed: benzophenone, clonazepam, diazepam, demoxepam, flurazepam

KEY WORDS

post-column reaction

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

ANNOTATED BIBLIOGRAPHY

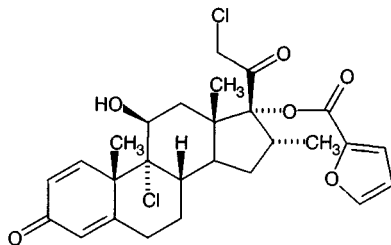
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- Ha, H.R.; Rentsch, K.M.; Kneer, J.; Vonderschmitt, D.J. Determination of midazolam and its alpha-hydroxy metabolite in human plasma and urine by high-performance liquid chromatography. *Ther.Drug Monit.*, **1993**, *15*, 338–343
- Noggle, F.T., Jr.; Clark, C.R.; DeRuiter, J. Liquid chromatographic separation of some common benzodiazepines and their metabolites. *J.Liq.Chromatogr.*, **1990**, *13*, 4005–4021 [also metabolites, alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, demoxepam, diazepam, estazolam, fludiazepam, flunitrazepam, flurazepam, halazepam, lorazepam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam, triazolam]
- Vasiliades, J.V.; Sahawneh, T. Midazolam determination by gas chromatography, liquid chromatography and gas chromatography-mass spectrometry. *J.Chromatogr.*, **1982**, *228*, 195–203
- Vasiliades, J.; Sahawneh, T.H. Determination of midazolam by high-performance liquid chromatography. *J.Chromatogr.*, **1981**, *225*, 266–271
- Vree, T.B.; Baars, A.M.; Booij, L.H.; Driessen, J.J. Simultaneous determination and pharmacokinetics of midazolam and its hydroxymetabolites in plasma and urine of man and dog by means of high-performance liquid chromatography. *Arzneimittelforschung*, **1981**, *31*, 2215–2219

Mometasone Furoate

Molecular formula: C₂₇H₃₀Cl₂O₆

Molecular weight: 521.4

CAS Registry No.: 83919-23-7



SAMPLE

Matrix: formulations

Sample preparation: Dissolve in MeCN:THF 94.3:5.7, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Suplex pKb-100 (Supelco)

Mobile phase: MeCN:THF:15 mM pH 4.1 acetate buffer 41.5:2.5:56

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Simultaneous: degradation products, impurities, benzaldehyde, benzyl alcohol, clotrimazole, orthochlorophenyl-diphenylmethanol

KEY WORDS

creams

REFERENCE

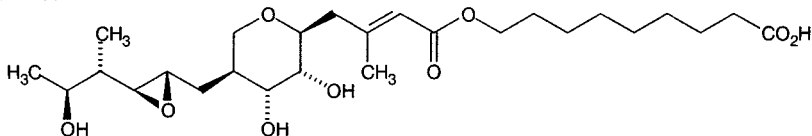
Spangler, M. Isocratic reversed phase HPLC analysis of a pharmaceutical cream. *Supelco Reporter*, 1994, 13(2), 12-13

Mupirocin

Molecular formula: C₂₆H₄₄O₉

Molecular weight: 500.6

CAS Registry No.: 12650-69-0



SAMPLE

Matrix: formulations

Sample preparation: 5 g Ointment + 100 mL MeCN + 300 mL 50 mM pH 6.3 phosphate buffer, mechanically stir for 30 min, make up to 500 mL with 50 mM pH 6.3 phosphate buffer, filter (0.2 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 5 Waters radial-pak C18

Mobile phase: MeCN:50 mM pH 6.3 phosphate buffer 25:75

Flow rate: 2

Injection volume: 20

Detector: UV 229

CHROMATOGRAM

Retention time: 5.5

KEY WORDS

ointments

REFERENCE

Jagota, N.K.; Stewart, J.T.; Warren, F.W.; John, P.M. Stability of mupirocin ointment (Bactroban) admixed with other proprietary dermatological products. *J.Clin.Pharm.Ther.*, **1992**, *17*, 181–184

SAMPLE

Matrix: nutrient broth

Sample preparation: Filter, mix an aliquot of the filtrate with MeCN, centrifuge at 2500 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 mm long MCH 5 Micro-Pak

Mobile phase: MeCN:water:orthophosphoric acid 30:70:1

Flow rate: 1

Injection volume: 10

Detector: UV 215

CHROMATOGRAM

Retention time: 18.9

OTHER SUBSTANCES

Noninterfering: metabolites

REFERENCE

Cookson, B. Failure of mupirocin-resistant staphylococci to inactivate mupirocin. *Eur.J.Clin. Microbiol.Infect.Dis.*, **1989**, *8*, 1038–1040

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeOH:50 mM pH 4.5 ammonium acetate 60:40

Flow rate: 1

CHROMATOGRAM

Retention time: 15

REFERENCE

Farmer, T.H.; Gilbert, J.; Elson, S.W. Biochemical basis of mupirocin resistance in strains of *Staphylococcus aureus*. *J.Antimicrob.Chemother.*, **1992**, *30*, 587–596

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeOH:50 mM pH 4.5 ammonium acetate 40:60

Flow rate: 2

Injection volume: 50

Detector: UV 235

KEY WORDS

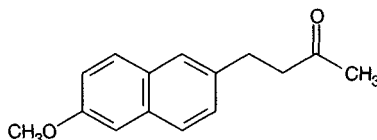
radiolabeled

REFERENCE

Morecombe, D.J. High-efficiency preparative-scale reversed-phase high-performance liquid chromatographic purification of 14C-labelled antibiotics. *J.Chromatogr.*, **1987**, *389*, 389–395

Nabumetone

Molecular formula: C₁₅H₁₆O₂
Molecular weight: 228.3
CAS Registry No.: 42924-53-8



SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 200 µL 200 µg/mL naproxen + 1 mL 500 mM pH 3 citrate buffer + 100 µL 1 M HCl + 5 mL ether, shake for 2 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in MeCN, inject an aliquot.

HPLC VARIABLES

Guard column: Resolve C18 Guard-Pak (Waters)

Column: C18 Radial-Pak (Waters)

Mobile phase: MeCN:sodium acetate buffer 60:40

Detector: UV 280

CHROMATOGRAM

Internal standard: naproxen

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Brier, M.E.; Sloan, R.S.; Aronoff, G.R. Population pharmacokinetics of the active metabolite of nabumetone in renal dysfunction. *Clin.Pharmacol.Ther.*, **1995**, *57*, 622-627

SAMPLE

Matrix: blood

Sample preparation: 0.5 mL Plasma + 2.5 µL 400 µg/mL 6-chloro-2-naphthylacetic acid in acetone, mix on a whirlmixer, add 6 mL n-hexane:ethyl acetate 50:50, add 0.7 mL 1.5 M HCl, shake mechanically for 30 min, centrifuge at 1500 g for 10 min. Remove organic layer and evaporate it to dryness at 37° under a stream of nitrogen. Dissolve residue in mobile phase, centrifuge, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: Lichrosorb RP-8

Column: 250 × 4.6 5 µm Ultrasphere ODS

Mobile phase: MeOH:50 mM pH 3.0 sodium acetate 70:30

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: F ex 284 em 320 (cut-off)

CHROMATOGRAM

Retention time: 7

Internal standard: 6-chloro-2-naphthylacetic acid (6)

Limit of detection: 250 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites**Simultaneous:** acetaminophen, aspirin, naproxen, salicylic acid

KEY WORDS

plasma

REFERENCE

Ray, J.E.; Day, R.O. High-performance liquid chromatographic determination of a new anti-inflammatory agent, nabumetone, and its major metabolite in plasma using fluorimetric detection. *J.Chromatogr.*, **1984**, *336*, 234–238

SAMPLE**Matrix:** blood, synovial fluid**Sample preparation:** 100 μ L Plasma or synovial fluid + 200 μ L 2 mg/mL IS in MeCN, vortex thoroughly, centrifuge at 11000 rpm for 5 min, inject an 80 μ L aliquot of the supernatant. Tissue. Homogenize (Ultra-Turrax) 150 mg tissue with 600 (synovial membrane) or 1200 (fibrous capsule tissue) mg water for 1 min, vortex vigorously, sonicate for 30 min, vortex, centrifuge. Remove a 50 μ L aliquot and add it to 100 μ L 1 (synovial membrane) or 0.33 (fibrous capsule tissue) mg/mL IS in MeCN, vortex thoroughly, centrifuge at 11000 rpm for 5 min, inject an 4 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** 150 \times 3.9 Resolve 5 C18 (Waters)**Mobile phase:** MeCN:50 mM NaH₂PO₄ 40:60, pH 5.2 (For the active metabolite, 6-methoxy-2-naphthylacetic acid, use MeCN:50 mM NaH₂PO₄ 30:70, pH 5.2.)**Flow rate:** 1.6**Injection volume:** 4-80**Detector:** F ex 224 em 356

CHROMATOGRAM**Internal standard:** BRL 24333**Limit of detection:** <21 ng/mL

KEY WORDS

plasma; synovial membrane; fibrous capsule tissue

REFERENCE

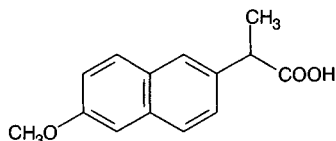
Miehlke, R.K.; Schneider, S.; Sörgel, F.; Muth, P.; Henschke, F.; Giersch, K.H.; Münzel, P. Penetration of the active metabolite of nabumetone into synovial fluid and adherent tissue of patients undergoing knee joint surgery. *Drugs*, **1990**, *40 Suppl 5*, 57–61

Naproxen

Molecular formula: C₁₄H₁₄O₃

Molecular weight: 230.3

CAS Registry No.: 22204-53-1, 26159-34-2 (sodium salt)



SAMPLE

Matrix: aqueous humor

Sample preparation: 100 μ L Aqueous humor + 500 μ L MeCN, 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 μ L 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 μ L under nitrogen.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m 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: 3.89

Internal standard: naproxen

OTHER SUBSTANCES

Extracted: diclofenac, flurbiprofen

Simultaneous: bacitracin, cortisone, diazepam, fluorometholone, hydrocortisone, imipramine, indomethacin, ketorolac, levobunolol, meclofenamic acid, metipranolol, neomycin, prednisolone, proracaine, 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

Interfering: ketoprofen

KEY WORDS

rabbit; human; naproxen is IS

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: 500 μ L Plasma + 500 μ L 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 μ L MeCN, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 5 5 μ m 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

OTHER SUBSTANCES

Extracted: diclofenac

KEY WORDS

plasma; naproxen is IS

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

Sample preparation: 500 μ L Plasma + 125 μ L 40 mM decanoic acid in MeCN, mix. Dialyze a 100 μ L sample against 20 mM pH 7.0 phosphate buffer using a Gilson Cuprophane membrane (molecular mass cut-off 15 kDa). Continuously pump the buffer through the dialysis cell and through column A at 3 mL/min for 9.6 min, backflush the contents of column A onto column B with the mobile phase, monitor the effluent from column B. (After each injection flush plasma channel with 1 mL 0.05% Triton X-100, with 1 mL 1 mM HCl, and with 2 mL water. After each injection flush buffer channel with 3 mL 20 mM pH 7.0 phosphate buffer and condition column A with 1 mL 20 mM pH 7.0 phosphate buffer.)

HPLC VARIABLES

Column: A 10 \times 2 40 μ m Bondesil C18 (Analytichem); B 250 \times 3.1 5 μ m C18 (RoSil Research Separation Laboratories)

Mobile phase: MeCN:MeOH:20 mM pH 3.2 phosphate buffer 50:10:40

Flow rate: 1

Injection volume: 100

Detector: UV 272

CHROMATOGRAM

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: fenoprofen (UV 272), flurbiprofen (UV 247), ibuprofen (UV 264), ketoprofen (UV 261)

KEY WORDS

plasma; dialysis; column-switching

REFERENCE

Herráez-Hernández, R.; Van de Merbel, N.C.; Brinkman, U.A.T. Determination of the total concentration of highly protein-bound drugs in plasma by on-line dialysis and column liquid chromatography: application to non-steroidal anti-inflammatory drugs. *J.Chromatogr.B*, **1995**, *666*, 127-137

SAMPLE

Matrix: blood

Sample preparation: Add 5 μL 42% phosphoric acid to 100 μL plasma and freeze until required. 100 μL Acidified plasma + 10 μL 10 $\mu\text{g}/\text{mL}$ homotryptophol in MeOH + 200 μL MeCN, vortex for 1 min, centrifuge, filter (0.45 μm), inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Ultrasphere ODS

Mobile phase: MeCN:50 mM pH 6.0 ammonium acetate 25:75

Flow rate: 1

Detector: F ex 275 em 345

CHROMATOGRAM

Retention time: 24.7

Internal standard: homotryptophol (22)

Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: metabolites, glucuronides

KEY WORDS

rat; plasma; pharmacokinetics

REFERENCE

Iwaki, M.; Bischer, A.; Nguyen, A.C.; McDonagh, A.F.; Benet, L.Z. Stereoselective disposition of naproxen glucuronide in the rat. *Drug Metab. Dispos.*, **1995**, 23, 1099–1103

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 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 $^{\circ}$ 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 \times 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: 5.8

Internal standard: naproxen

OTHER SUBSTANCES

Extracted: diclofenac

KEY WORDS

plasma; naproxen is IS

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:** 500 μ L Plasma + 20 μ L 500 μ g/mL phenylbutazone in MeOH + 1.5 mL MeOH, vortex, centrifuge for 15 min at 3000 g. Remove the supernatant and evaporate it to 500 μ L using a vortex evaporator, inject a 20 μ L aliquot.

HPLC VARIABLES**Guard column:** present but not specified**Column:** 10 μ m RP-8 (Alltech)**Mobile phase:** MeOH:1% acetic acid 70:30**Injection volume:** 20**Detector:** UV 240

CHROMATOGRAM**Internal standard:** phenylbutazone**Limit of quantitation:** 100 ng/mL

OTHER SUBSTANCES**Extracted:** napdice

KEY WORDS

dog; plasma; pharmacokinetics

REFERENCESamara, E.; Avnir, D.; Ladkani, D.; Bialer, M. Pharmacokinetic analysis of diethylcarbonate prodrugs of ibuprofen and naproxen. *Biopharm. Drug Dispos.*, **1995**, *16*, 201–210

SAMPLE**Matrix:** blood**Sample preparation:** 200 μ L Serum + 20 μ L 1 mg/mL phenyl salicylate in mobile phase, vortex briefly, add 560 μ L MeCN slowly with continuous vortexing, vortex for 30 s, centrifuge at 3500 rpm for 10 min, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES**Guard column:** 30 μ m Perisorb C18**Column:** 250 \times 4.6 5 μ m Partisil ODS-3 C18**Mobile phase:** MeCN:40 mM pH 2.5 HCl buffer 47:53**Flow rate:** 1.2**Injection volume:** 50**Detector:** F ex 230 em 370

CHROMATOGRAM**Retention time:** 5**Internal standard:** phenyl salicylate (10)**Limit of detection:** 1 ng/mL**Limit of quantitation:** 2 ng/mL

KEY WORDS

serum; dog; pharmacokinetics

REFERENCESuh, H.; Jun, H.W.; Lu, G.W. Fluorometric high performance liquid chromatography for quantitation of naproxen in serum. *J. Liq. Chromatogr.*, **1995**, *18*, 3105–3115

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)) KH₂PO₄ 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: 5.34

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, caffeine, carbamazepine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacine, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenopropfen, fentiazac, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metoclopramide, metoprolol, mexiletine, mianserine, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, penbutolol, penfluridol, pentazocine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfipyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thioproperazine, thioridazine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluperidol, trimipramine, trospatenine, viloxazine, vinblastine, vincristine, vindesine, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: bupivacaine, buprenorphine, buspirone, carbinoxamine, clorazepate, fenfluramine, flecainide, loprazolam, metipranolol, midazolam, oxprenolol, phencyclidine, thio-pental, tianeptine, triprolidine, verapamil, warfarin

KEY WORDSwhole blood; plasma

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 μ L Plasma + 50 μ L 1 M HCl + 1 mL 20 μ g/mL indomethacin in chloroform, extract. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 2 mL MeCN:water 50:50, inject a 50 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeCN:water:orthophosphoric acid 50:49.5:0.5**Flow rate:** 1**Injection volume:** 50**Detector:** UV 230

CHROMATOGRAM**Retention time:** 5.1**Internal standard:** indomethacin (9.4)**Limit of quantitation:** 1.5 μ g/mL

KEY WORDSplasma; pharmacokinetics

REFERENCE

Charles, B.G.; Mogg, G.A.G Comparative *in vitro* and *in vivo* bioavailability of naproxen from tablet and caplet formulations. *Biopharm. Drug Dispos.*, **1994**, *15*, 121–128

SAMPLE**Matrix:** blood**Sample preparation:** Inject sample onto column A with mobile phase A and elute for 3 min. Backflush contents of column A onto column B with mobile phase B for 4 min and elute column B with mobile phase B and monitor eluant.

HPLC VARIABLES**Column:** A 10 \times 3 BioTrap Acid C18 (ChromTech); B 10 \times 3 CT-sil C18 guard column + 100 \times 4.6 5 μ m CT-sil C18 (ChromTech)**Mobile phase:** A 200 mM pH 2.1 phosphate buffer; B MeOH:120 mM pH 3.0 phosphate buffer 65:35**Flow rate:** A 0.55; B 1**Injection volume:** 50**Detector:** UV 328

CHROMATOGRAM**Retention time:** 6.5**Limit of quantitation:** 1050 ng/mL

KEY WORDS

plasma; column-switching; direct injection

REFERENCE

Hermansson, J.; Grahn, A. Determination of drugs by direct injection of plasma into a biocompatible extraction column based on a protein-entrapped hydrophobic phase. *J.Chromatogr.A*, **1994**, *660*, 119-129

SAMPLE

Matrix: blood

Sample preparation: 25 μ L Plasma + 100 μ L 10 μ g/mL naproxen in MeCN, vortex 30 s, centrifuge at 11000-12300 g for 7 min. Remove supernatant and evaporate it under air at 55°. Dissolve residue in 50 μ L mobile phase and inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:80 mM pH 2.0 orthophosphoric acid 46:54

Flow rate: 1.1

Injection volume: 20

Detector: F ex 270 em 410

CHROMATOGRAM

Retention time: 11.5

Internal standard: naproxen

OTHER SUBSTANCES

Extracted: furosemide

Noninterfering: amikacin, amoxicillin, dexamethasone, gentamicin, indomethacin, morphine, phenobarbital, theophylline, vitamins

KEY WORDS

plasma; microscale; neonatal; naproxen is IS

REFERENCE

Sidhu, J.S.; Charles, B.G. Simple microscale high-performance liquid chromatographic method for determination of furosemide in neonatal plasma. *J.Chromatogr.*, **1993**, *612*, 161-165

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 200 μ L 2 M HCl + 6 mL hexane:diethyl ether 8:2 (ice cold), extract, centrifuge at 1500 g for 5 min. Remove 5 mL of organic layer and evaporate it to dryness under a stream of nitrogen, redissolve in 500 μ L 30 mM pH 7.5 phosphate buffer, inject 50-100 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4 3 μ m Nucleosil RP 8

Mobile phase: MeCN:water 40:60, acidified with 1 mL 85% phosphoric acid

Flow rate: 1

Injection volume: 50-100

Detector: UV 246

CHROMATOGRAM

Retention time: 4.2

Internal standard: S-naproxen

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: flurbiprofen

KEY WORDS

plasma; naproxen is IS

REFERENCE

Giesslinger, G.; Menzel-Soglowek, S.; Schuster, O.; Brune, K. Stereoselective high-performance liquid chromatographic determination of flurbiprofen in human plasma. *J.Chromatogr.*, **1992**, *573*, 163–167

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 500 μ L 2 M sulfuric acid + 8 mL n-hexane:ethyl acetate 90:10, mix gently at 30 rpm for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and evaporate it to dryness at 45° under a stream of nitrogen. Reconstitute in 100 μ L 1.5% thionyl chloride in n-hexane (freshly prepared), heat at 75° for 1 h in a capped tube, cool to room temperature, add 500 μ L 2% S-1-phenylethylamine in dichloromethane (freshly prepared), let stand for 15 min, add 500 μ L 2 M sulfuric acid + 5 mL n-hexane, mix gently at 30 rpm for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and evaporate it to dryness at 45° under a stream of nitrogen. Reconstitute in 250 μ L mobile phase, inject 200 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 μ m SGE silica glass column

Mobile phase: n-Heptane:isopropanol 92:8

Flow rate: 1

Injection volume: 200

Detector: UV 254

CHROMATOGRAM

Retention time: 5.9

Internal standard: S-naproxen

Limit of quantitation: 150 ng/mL

OTHER SUBSTANCES

Extracted: ketoprofen

Simultaneous: fenoprofen, ibuprofen, mefenamic acid, salicylic acid

Noninterfering: diazepam, digoxin, methylprednisolone, midazolam, nifedipine, penicillamine, ranitidine, theophylline

KEY WORDS

plasma; normal phase; naproxen is IS; derivatization; chiral

REFERENCE

Hayball, P.J.; Nation, R.L.; Bochner, F.; Le Leu, R.K. Enantiospecific analysis of ketoprofen in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, *570*, 446–452

SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH, 5 mL water, and 1 mL buffer. 20–200 μ L Plasma + 100 μ L MeOH + 20 μ L 50 μ g/mL indomethacin in MeOH + 100 μ L buffer + 100 μ L water, vortex for 2 min, centrifuge at 1800 g for 10 min, apply supernatant to the SPE cartridge, wash with 5 mL water, elute with two 5 mL portions of MeOH, evaporate eluate and take up residue in 1 mL mobile phase. (Buffer was 250 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)

HPLC VARIABLES

Column: 100 × 4.6 5 μm Brownlee RP18

Mobile phase: MeOH:25 mM pH 3.0 phosphate buffer 75:25 (Prepare buffer by diluting a 250 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: 10.0

Internal standard: indomethacin (14.6)

Limit of detection: 15 ng/mL

OTHER SUBSTANCES

Extracted: diflunisal, sulindac

KEY WORDS

plasma; SPE

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

Sample preparation: Dog, rat. 100 μL Plasma + 100 μL 1 M HCl + 6 mL MTBE, vortex for 1 min, centrifuge at 1200 g for 2 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35°. Reconstitute in 1 mL 2% MeOH, vortex for 2 min, inject a 100 μL aliquot. Human. 500 μL Plasma + 200 μL 1 M HCl + 8 mL diethyl ether, vortex for 1 min, centrifuge at 1200 g for 2 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35°. Reconstitute in 2 mL 2% MeOH, vortex for 2 min, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Spherisorb C8

Mobile phase: MeCN:THF:MeOH:2% acetic acid (pH 2.5) 5:18:5:72

Flow rate: 1.5

Injection volume: 100

Detector: UV 330

CHROMATOGRAM

Retention time: 8

Internal standard: naproxen

OTHER SUBSTANCES

Extracted: 5'-hydroxypiroxicam, piroxicam

KEY WORDS

plasma; dog; rat; human; naproxen is IS

REFERENCE

Gillilan, R.B.; Mason, W.D.; Fu, C.-H.J. Rapid analysis of piroxicam in dog, rat and human plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1989**, 487, 232–235

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 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: 3.5
Internal standard: naproxen

OTHER SUBSTANCES

Extracted: bumetanide, furosemide

KEY WORDS

plasma; naproxen is IS; horse

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: Condition a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply to the SPE cartridge. Wash with 100 µL water, elute with three 500 µL portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 µm Spherisorb ODS
Mobile phase: MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Extracted: acetaminophen, fenoprofen, ibuprofen, indomethacin, ketoprofen, salicylic acid

KEY WORDS

whole blood; SPE

REFERENCE

Moore, C.M.; Tebbett, I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis. *Forensic Sci.Int.*, 1987, 34, 155-158

SAMPLE**Matrix:** blood**Sample preparation:** Centrifuge serum at 15° at 203000 g for 20 h, remove 1 mL of the supernatant and add 250 µL 4 M HCl, add 5 mL distilled diethyl ether, rotate at 30 rpm for 15 min, centrifuge at 700 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 the whole amount.

HPLC VARIABLES**Column:** µBondapak C18 Radial Pak**Mobile phase:** MeCN:100 mM (NH₄)H₂PO₄ 63:37**Flow rate:** 1.5**Injection volume:** 100**Detector:** F ex 320 em 390 following post-column reaction. The column effluent mixed with 12% triethanolamine (for fluorescence enhancement) pumped at 0.5 mL/min and flowed through a 1 m reaction coil to the detector.

CHROMATOGRAM**Internal standard:** naproxen

OTHER SUBSTANCES**Extracted:** warfarin**Simultaneous:** salicylic acid**Noninterfering:** acetaminophen, carbamazepine, furosemide, hydrochlorothiazide, phenobarbital, phenytoin, spironolactone

KEY WORDS

serum; post-column reaction; ultracentrifugate; naproxen is IS

REFERENCESteyn, J.M.; van der Merwe, H.M.; de Kock, M.J. Reversed-phase high-performance liquid chromatographic method for the determination of warfarin from biological fluids in the low nanogram range. *J.Chromatogr.*, **1986**, *378*, 254–260

SAMPLE**Matrix:** blood**Sample preparation:** 500 µL Plasma + 200 µL, 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:** 2.55**Internal standard:** naproxen**Limit of detection:** <5 µg/mL

OTHER SUBSTANCES

Extracted: diflunisal

KEY WORDS

plasma; naproxen is IS

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, synovial fluid

Sample preparation: 0.5 mL Plasma or synovial fluid + 50 μ L 10 mg/mL diphenylacetic acid + 200 μ L 2 M HCl + 5 mL diethyl ether, tumble 10 min on a rotary mixer. Remove organic layer and evaporate it to dryness under vacuum centrifugation. Reconstitute residue in 500 μ L mobile phase, inject aliquot.

HPLC VARIABLES

Guard column: 20 \times 2 Perisorb RP18 30-40 μ m pellicular

Column: 125 \times 4.6 5 μ m Spherisorb ODS 1

Mobile phase: MeOH:Sorensen's phosphate buffer (pH 7) 37:63

Flow rate: 1

Injection volume: 20

Detector: UV 260

CHROMATOGRAM

Retention time: 8

Internal standard: diphenylacetic acid (5)

Limit of detection: <5 μ g/mL

KEY WORDS

plasma

REFERENCE

Blagbrough, I.S.; Daykin, M.M.; Doherty, M.; Patrick, 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: Acidify plasma or urine with 1 M pH 2 phosphate buffer, extract with hexane:THF 80:20.

HPLC VARIABLES

Guard column: 20 \times 4.6 Nucleosil OCS 10

Column: 150 \times 6 YMC Pack A312 S5 120A ODS

Mobile phase: MeCN:50 mM phosphate buffer 19:83

Column temperature: 28

Flow rate: 2

Injection volume: 50

Detector: UV 262

CHROMATOGRAM**Retention time:** 11.11 (plasma), 10.47 (urine)**Internal standard:** naproxen

OTHER SUBSTANCES**Extracted:** ketoprofen

KEY WORDSplasma; rat; naproxen is IS

REFERENCE

Daffonchio, L.; Bestetti, A.; Clavenna, G.; Fedele, G.; Ferrari, M.P.; Omini, C. Effect of a new foam formulation of ketoprofen lysine salt in experimental models of inflammation and hyperalgesia. *Arzneimittelforschung*, **1995**, *45*, 590–594

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 1 mL Plasma + 1 mL 100 mM HCl + 10 mL dichloromethane, rotate for 10 min, centrifuge at 1500 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in mobile phase, inject a 20 µL aliquot. Urine. 50 µL Urine + 1 mL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES**Column:** 75 × 4.6 3 µm Supelcosil LC-8**Mobile phase:** MeCN:50 mM phosphoric acid 45:55**Flow rate:** 1**Injection volume:** 20**Detector:** F ex 235 em 405; UV 235

CHROMATOGRAM**Retention time:** 3.2**Limit of detection:** 20-50 ng/mL (F)

OTHER SUBSTANCES**Extracted:** ethacrynic acid, indomethacin, mefenamic acid, phenylbutazone, thiosalicylic acid**Interfering:** flunixin (with UV detection not with F detection)

KEY WORDSplasma

REFERENCE

Singh, A.K.; Jang, Y.; Mishra, U.; Granley, K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicylic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry. *J.Chromatogr.*, **1991**, *568*, 351–361

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 350 µL 10 µg/mL Ketoprofen in 10 mM pH 6.0 phosphate buffer containing 0.1% MeOH + 650 µL pH 6 phosphate buffer + 1 mL plasma or urine + 0.5 mL 1 M pH 2 (plasma) or 1 mL 0.5 M pH 7 (urine) phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and

evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μ L mobile phase, vortex for 15 s, inject aliquot.

HPLC VARIABLES

Guard column: 40 \times 3.2 30-44 μ m Vydac reverse-phase

Column: 40 \times 4.6 5 μ m Spherisorb ODS

Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 6:94 to 8:92

Flow rate: 2

Injection volume: 5-200

Detector: UV 262

CHROMATOGRAM

Retention time: 10

Internal standard: ketoprofen (16)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, fenoprofen, probenecid, salicylic acid

KEY WORDS

plasma

REFERENCE

Upton, R.A.; Buskin, J.N.; Guentert, T.W.; Williams, R.L.; Riegelman, S. Convenient and sensitive high-performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine. *J.Chromatogr.*, **1980**, *190*, 119-128

SAMPLE

Matrix: bulk

Sample preparation: 10 mg Compound + 10 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride + 2 drops 3,5-dimethylaniline + 1.5 mL dichloromethane, mix, after 30 min add 1 mL 1 M HCl, shake vigorously. Remove the lower organic layer and dry it over anhydrous magnesium sulfate, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m D N-(3,5-dinitrobenzoyl)phenylglycine (Regis)

Mobile phase: Hexane:isopropanol 80:20

Flow rate: 2

Injection volume: 20

Detector: UV 254; UV 280

CHROMATOGRAM

Retention time: k' 9.10 (for first enantiomer)

OTHER SUBSTANCES

Also analyzed: carprofen, cicloprofen, etodolac, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, pirprofen, tiaprofenic acid

KEY WORDS

derivatization; $\alpha = 1.17$; chiral

REFERENCE

Pirkle, W.H.; Murray, P.G. The separation of the enantiomers of a variety of non-steroidal anti-inflammatory drugs (NSAIDs) as their anilide derivatives using a chiral stationary phase. *J.Liq.Chromatogr.*, **1990**, *13*, 2123-2134

SAMPLE**Matrix:** dialysate**Sample preparation:** Inject a 10 μ L aliquot of dialysate (pH 7.4 isotonic phosphate buffer).

HPLC VARIABLES**Guard column:** 37-50 μ m Corasil C18**Column:** 100 \times 4.5 μ m Nucleosil C18**Mobile phase:** MeCN:50 mM pH 3.0 phosphate buffer 48:52**Flow rate:** 1.1**Injection volume:** 10**Detector:** F ex 262 em 356

CHROMATOGRAM**Retention time:** 2.5**Internal standard:** naproxen

OTHER SUBSTANCES**Extracted:** flurbiprofen (F ex 258 em 310)

KEY WORDS

mouse; rat; naproxen is IS

REFERENCEEvrard, P.A.; Deridder, G.; Verbeeck, R.K. Intravenous microdialysis in the mouse and the rat: Development and pharmacokinetic application of a new probe. *Pharm.Res.*, **1996**, *13*, 12-17

SAMPLE**Matrix:** formulations**Sample preparation:** Dissolve in 100 mM pH 6-8 phosphate buffer (total ionic strength 0.5), inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 μ m Supelcosil C18**Mobile phase:** MeCN:50 mM phosphate buffer 25:75**Flow rate:** 1**Detector:** UV 222

CHROMATOGRAM**Retention time:** 4.2

KEY WORDS

tablets

REFERENCEChakrabarti, S.; Southard, M.Z. Control of poorly soluble drug dissolution in conditions simulating the gastrointestinal tract flow. 1. Effect of tablet geometry in buffered medium. *J.Pharm.Sci.*, **1996**, *85*, 313-319

SAMPLE**Matrix:** formulations**Sample preparation:** Dissolve tablet in 10 mM HCl containing 90 mM KCl (pH 2.0), inject an aliquot.

HPLC VARIABLES**Column:** 50 mm long ODS Hypersil C18

Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 32:68 containing 5 mM tetrabutylammonium

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 6.0

OTHER SUBSTANCES

Simultaneous: phenytoin

KEY WORDS

tablets

REFERENCE

Neervannan, S.; Dias, L.S.; Southard, M.Z.; Stella, V.J. A convective-diffusion model for dissolution of two non-interacting drug mixtures from co-compressed slabs under laminar hydrodynamic conditions. *Pharm.Res.*, **1994**, *11*, 1288–1295

SAMPLE

Matrix: solutions

Sample preparation: Filter (0.45 μm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:10 mM pH 6 phosphate buffer 52:48

Detector: UV 250

REFERENCE

Okimoto, K.; Rajewski, R.A.; Uekama, K.; Jona, J.A.; Stella, V.J. The interaction of charged and uncharged drugs with neutral (HP- β -CD) and anionically charged (SBE7- β -CD) β -cyclodextrins. *Pharm.Res.*, **1996**, *13*, 256–264

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 \times 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: 5.6

Limit of quantitation: 200-500 ng/mL

OTHER SUBSTANCES

Simultaneous: acemetacin, diclofenac, flurbiprofen, indomethacin, ketoprofen, lonazolac, 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: 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: 6.97 (A), 7.18 (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, hydroxyzine, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, 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, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimepazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica**Mobile phase:** Heptane:isopropanol:trifluoroacetic acid 98:2:0.1**Flow rate:** 1**Injection volume:** 1000**Detector:** UV 254

CHROMATOGRAM**Retention time:** k' 4.48

KEY WORDSchiral; $\alpha = 1.16$

REFERENCEOliveros, 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:** 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, dipyrrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropofen, 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, isocarboxystiril, isocarboxazid, isoniazid, isoproter-

enol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, 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, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 Chiralpak AD (Daicel)

Mobile phase: Carbon dioxide:MeOH 96:4

Column temperature: 30

Flow rate: 2.5

Detector: UV 210

CHROMATOGRAM

Retention time: 18.5, 23 (enantiomers)

OTHER SUBSTANCES

Simultaneous: fenoprofen, flurbiprofen, ibuprofen, ketoprofen

KEY WORDS

SFC; 250 bar; chiral

REFERENCE

Kot, A.; Sandra, P.; Venema, A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs. *J.Chromatogr.Sci.*, **1994**, *32*, 439-448

SAMPLE**Matrix:** solutions**Sample preparation:** Inject an aliquot of a 50 $\mu\text{g/mL}$ solution.

HPLC VARIABLES**Column:** 100 \times 4.6 Chiral AGP CSP (ChromTech)**Mobile phase:** Isopropanol:4 mM pH 7.0 phosphate buffer 0.5:99.5**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 263

CHROMATOGRAM**Retention time:** 3 (R), 4.5. (S)

REFERENCEKern, J.R. Chromatographic separation of the optical isomers of naproxen. *J.Chromatogr.*, **1991**, *543*, 355-306

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 50 μL aliquot of a solution in mobile phase.

HPLC VARIABLES**Column:** 100 \times 4.6 5 μm Spheri-5 RP-8**Mobile phase:** MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na_2HPO_4 and 7 mM KH_2PO_4 to achieve pH 7.)**Flow rate:** 1**Injection volume:** 50**Detector:** F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 $\mu\text{g/mL}$ reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m \times 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylenebacked 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α -(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetoneitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α -(3,4-dimethoxyphenyl)-4'-methylcinnamonitrile. Dissolve 20 mmoles α -(3,4-dimethoxyphenyl)-4'-methylcinnamonitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h, cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α -(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α -(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α -(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile (*J.Chem.Eng.Data* 1987, 32, 387). Reflux 10 mmoles α -(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α -(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM**Retention time:** k' 0.9690**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES**Simultaneous:** ibuprofen, ketoprofen, mefenamic acid, probenecid, salicylic acid, valproic acid

KEY WORDS

post-column extraction

REFERENCE

Kim, M.; Stewart, J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α -phenylcinnamionitrile quaternary ammonium salt as a new fluorescent ion-pair reagent. *J.Liq.Chromatogr.*, **1990**, *13*, 213–237

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 \times 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:** 12

OTHER SUBSTANCES**Simultaneous:** aspirin, carprofen, diflunisal, fenbufen, ibuprofen, indomethacin, tolmetin

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

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Terfloth, G.J.; Pirkle, W.H.; Lynam, K.G.; Nicolas, E.C. Broadly applicable polysiloxane-based chiral stationary phase for high-performance liquid chromatography and supercritical fluid chromatography. *J.Chromatogr.A*, **1995**, *705*, 185–194 [chiral; SFC; HPLC; also carprofen, cicloprofen, etodolac, fenoprofen, flurbiprofen, ibuprofen, piroprofen, warfarin]

Kempe, M.; Mosbach, K. Direct resolution of naproxen on a non-covalently molecularly imprinted chiral stationary phase. *J.Chromatogr.A*, **1994**, *664*, 276–279 [chiral]

Szász, G.; Budvári-Bárany, Z.; Löre, A.; Radecky, G.; Shalaby, A. HPLC of antiphlogistic acids on silica dynamically modified with cetylpyridinium chloride. *J.Liq.Chromatogr.*, **1993**, *16*, 2335–2345 [also diclofenac, fenoprofen, ibuprofen, ketoprofen, nicotinic acid, niflumonic acid, salicylic acid]

Tsai, S.-W.; Wei, H.-J. Self-normalized analysis of lipase-catalyzed conversion of naproxen enantiomers. *J.Liq.Chromatogr.*, **1993**, *16*, 2993–3001 [chiral; normal phase; naphthalene (IS)]

Andersen, J.V.; Hansen, S.H. Simultaneous quantitative determination of naproxen, its metabolite 6-O-desmethylnaproxen and their five conjugates in plasma and urine samples by high-performance liq-

- uid chromatography on dynamically modified silica. *J.Chromatogr.*, **1992**, *577*, 325–333 [extracted metabolites; plasma; urine]
- Pirkle, W.H.; Welch, C.J.; Lamm, B. Design, synthesis, and evaluation of an improved enantioselective naproxen selector. *J.Org.Chem.*, **1992**, *57*, 3854–3860 [chiral; also carprofen, cicloprofen, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, piroprofen, tiaprofenic acid]
- Pirkle, W.H.; Welch, C.J. An improved chiral stationary phase for the chromatographic separation of underivatized naproxen enantiomers. *J.Liq.Chromatogr.*, **1992**, *15*, 1947–1955 [chiral; also cicloprofen, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, piroprofen, tiaprofenic acid]
- Vree, T.B.; Van den Biggelaar-Martea, M.; Verwey-Van Wissen, C.P.W.G.M. Determination of naproxen and its metabolite O-demethylnaproxen with their acyl glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatography. *J.Chromatogr.*, **1992**, *578*, 239–249 [plasma; urine; extracted metabolites; gradient; LOQ 1.5 µg/mL (plasma); LOQ 1 µg/mL (urine); pharmacokinetics]
- Pirkle, W.H.; Welch, C.J. Chromatographic separation of underivatized naproxen enantiomers. *J.Liq.Chromatogr.*, **1991**, *14*, 3387–3396 [chiral]
- Buszewski, B.; El Mouelhi, M.; Albert, K.; Bayer, E. Influence of the structure of chemically bonded C18 phases on HPLC separation of naproxen glucuronide diastereoisomers. *J.Liq.Chromatogr.*, **1990**, *13*, 505–524
- Lafontaine, D.; Mailhot, C.; Vermeulen, M.; Bissonnette, B.; Lambert, C. Influence of chewable sucralfate or a standard meal on the bioavailability of naproxen. *Clin.Pharm.*, **1990**, *9*, 773–777 [pharmacokinetics; LOQ 1 µg/mL; diphenylacetic acid (IS)]
- Wanwimolruk, S. A simple isocratic high-performance liquid chromatographic (HPLC) determination of naproxen in human plasma using a microbore column technique. *J.Liq.Chromatogr.*, **1990**, *13*, 1611–1625 [plasma; microbore; flurbiprofen (IS); fluorescence detection; LOD 100 ng/mL; interfering ephedrine; non-interfering acetaminophen, aspirin, caffeine, diazepam, diclofenac, indomethacin, lidocaine, lorazepam, metoprolol, phenylbutazone, phenytoin, pindolol, piroxicam, salicylic acid, salicylic acid, sulfanilamide, sulfapyrazole, theophylline, triazolam]
- Bui, K.H.; French, S.B. Direct serum injection and analysis of drugs with aqueous mobile phases containing triethylammonium acetate. *J.Liq.Chromatogr.*, **1989**, *12*, 861–873 [direct injection; plasma; dog; rat; fluorescence detection; UV detection; also antipyrine, atenolol, hexanophenone, metoprolol, propranolol]
- Pettersson, C.; Gioeli, C. Improved resolution of enantiomers of naproxen by the simultaneous use of a chiral stationary phase and a chiral additive in the mobile phase. *J.Chromatogr.*, **1988**, *435*, 225–228 [chiral]
- Satterwhite, J.H.; Boudinot, F.D. High-performance liquid chromatographic determination of ketoprofen and naproxen in rat plasma. *J.Chromatogr.*, **1988**, *431*, 444–449 [ketoprofen (IS); LOD 5 ng/mL; pharmacokinetics]
- Hermansson, J.; Erikson, M. Direct liquid chromatographic resolution of acidic drugs using a chiral α 1-acid glycoprotein column (Enantiopac). *J.Liq.Chromatogr.*, **1986**, *9*, 621–639 [chiral; also bendroflumethiazide, disopyramide, ethotoin, hexobarbital, ibuprofen, ketoprofen, 2-phenoxypropionic acid, RAC 109]
- Hylandskjaer, A.; Aarbakke, J. An improved microscale HPLC assay for naproxen plasma levels. *Acta Pharmacol.Toxicol.(Copenh)*, **1983**, *52*, 78–80
- Meinsma, D.A.; Radzik, D.M.; Kissinger, P.T. Determination of common analgesics in serum and urine by liquid chromatography/electrochemistry. *J.Liq.Chromatogr.*, **1983**, *6*, 2311–2335 [electrochemical detection; also acetaminophen, codeine, methyl salicylate, phenacetin, salicylic acid]
- Shimek, J.L.; Rao, N.G.; Khalil, S.K. An isocratic high-pressure liquid chromatographic determination of naproxen and desmethylnaproxen in human plasma. *J.Pharm.Sci.*, **1982**, *71*, 436–439
- van Loenhout, J.W.A.; van Ginneken, C.A.M.; Ketelaars, H.C.J.; Kimenai, P.M.; Tan, Y.; Gribnau, F.W.J. A high performance liquid chromatographic method for the quantitative determination of naproxen and des-methyl-naproxen in biological samples. *J.Liq.Chromatogr.*, **1982**, *5*, 549–561 [fluorescence detection; plasma; urine; extracted metabolites; column temp 35]
- Broquaire, M.; Rovei, V.; Braithwaite, R. Quantitative determination of naproxen in plasma by a simple high-performance liquid chromatographic method. *J.Chromatogr.*, **1981**, *224*, 43–49
- Burgoyne, R.F.; Brown, P.R.; Kaplan, S.R. The simultaneous assay of naproxen and salicylic acid in serum using high pressure liquid chromatography. *J.Liq.Chromatogr.*, **1980**, *3*, 101–111 [fluorescence detection]

Neomycin

Molecular formula: C₂₃H₄₆N₆O₁₃ (neomycin B)

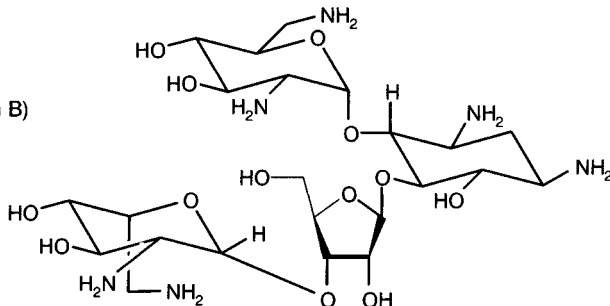
Molecular weight: 614.7 (neomycin B)

CAS Registry No.: 1404-04-2 (neomycin),

1406-04-8 (neomycin undecylenate),

1405-10-3 (neomycin sulfate),

1405-12-5 (neomycin palmitate)



SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μ L 30% trichloroacetic acid in water, vortex, centrifuge at 4° at 3600 g for 30 min. Remove 450 μ L of the supernatant and add it to 50 μ L buffer, vortex, centrifuge at 4° at 3600 g for 30 min, inject a 5-25 μ L aliquot of the supernatant. Urine. Centrifuge urine at 4° at 3600 g for 30 min. Remove a 180 μ L aliquot and add it to 20 μ L buffer, vortex, inject a 5-25 μ L aliquot. (Buffer contained 110 mM sodium 1-pentanesulfonate and 70 mM acetic acid.)

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Supelguard LC-8-DB (Supelco)

Column: 150 \times 4.6 5 μ m Supelcosil LC-8-DB

Mobile phase: MeOH:buffer 1.5:98.5 (Buffer contained 11 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)

Column temperature: 32.5

Injection volume: 5-30

Detector: F ex 340 em 455 following post-column derivatization. The column effluent mixed with an o-phthalaldehyde reagent (Pierce) and flowed through a reaction coil (PCR 520, Applied Biosystems) at 33° to the detector.

CHROMATOGRAM

Retention time: 16

Limit of quantitation: 250 ng/mL (plasma); 1000 ng/mL (urine)

KEY WORDS

plasma; cow; pharmacokinetics; post-column reaction

REFERENCE

Shaikh, B.; Jackson, J.; Guyer, G.; Ravis, W.R. Determination of neomycin in plasma and urine by high-performance liquid chromatography. Application to a preliminary pharmacokinetic study. *J. Chromatogr.*, **1991**, *571*, 189-198

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 20 mg neomycin sulfate powder in 100 mL 100 mM pH 8.0 sodium phosphate buffer. Remove a 10 mL aliquot and add it to 10 mL 40 mg/mL 2-naphthalenesulfonyl chloride in MeCN (prepare fresh daily), shake briefly, heat at 100-105° for 10 min, cool to room temperature, add 15 mL IS solution, shake vigorously for 10 min, centrifuge at <300 g for 3-5 min, inject a 50 μ L aliquot of the lower organic layer. (Prepare IS solution by dissolving 2 mg prednisolone in a small amount of THF, make up to 100 mL with chloroform.)

HPLC VARIABLES

Column: 125 \times 4.6 5 μ m P-E HS-5 silica (Perkin-Elmer)

Mobile phase: Chloroform:MeOH:acetic acid 95:2.3:2.5

Flow rate: 1.7

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 6.2 (neomycin B), 8.3 (neomycin C)

Internal standard: prednisolone (12.3)

OTHER SUBSTANCES

Simultaneous: impurities, neamine

KEY WORDS

derivatization; normal phase

REFERENCE

Tsuji, K.; Jenkins, K.M. Derivatization of primary amines by 2-naphthalenesulfonyl chloride for high-performance liquid chromatographic assay of neomycin sulfate. *J.Chromatogr.*, **1986**, 369, 105-115

SAMPLE

Matrix: formulations

Sample preparation: 5 g Ointment + 3 mL MeOH, heat at 55° for 5 min, vortex twice for 20 s, centrifuge at 2000 g for 2 min, discard the supernatant, repeat the MeOH wash twice more, add 30 mL chloroform, heat at 55°, vortex for 15 s, add 10 mL MeOH:water 20:80, shake vigorously for 20 min, centrifuge at 1000 g for 3 min, remove the upper aqueous layer, repeat the MeOH/water extraction twice more. Combine the aqueous layers and make up to 50 mL with 20 mM pH 9.0 borate buffer. Remove a 10 mL aliquot and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, inject an aliquot of the yellow organic layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb silica SI-100

Mobile phase: THF:chloroform:water:glacial acetic acid 39.2:59.8:0.8:0.2

Flow rate: 1

Injection volume: 40

Detector: UV 254

CHROMATOGRAM

Retention time: 4 (neomycin C), 12 (neomycin B)

OTHER SUBSTANCES

Simultaneous: hydrocortisone acetate

Noninterfering: cortisone acetate, fluorometholone, methylprednisolone, prednisolone acetate

KEY WORDS

normal phase; ointment; derivatization

REFERENCE

Binns, R.B.; Tsuji, K. High-performance liquid chromatographic analysis of neomycin in petrolatum-based ointments and in veterinary formulations. *J.Pharm.Sci.*, **1984**, 73, 69-72

SAMPLE

Matrix: formulations

Sample preparation: Powder. Dissolve 50 mg powder in 25 mL 20 mM pH 9.0 borate buffer, remove a 5 mL aliquot and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH (prepare fresh daily), heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, discard the upper aqueous phase, inject a 20 μ L aliquot of the lower organic phase. Ointment. Weigh out 5 g ointment, add 25 mL chloroform, heat at 60° until the ointment dissolved, shake vigorously for 15 min, centrifuge at 4000 g for 15 min, discard the chloroform, add 25 mL chloroform to the solid, centrifuge, discard the chloroform, add 10 mL 20 mM pH 9.0 borate buffer and 15 mL n-heptane to the solid, shake vigorously for 10 min, remove a 5 mL aliquot of the aqueous phase and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH (prepare fresh daily), heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, discard the upper aqueous phase, inject a 20 μ L aliquot of the lower organic phase.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m LiChrosorb SI-100

Mobile phase: Chloroform:THF:water 60:39.2:0.8

Flow rate: 1

Injection volume: 20

Detector: UV 350

CHROMATOGRAM

Retention time: 10 (neomycin C), 14 (neomycin B)

Limit of detection: 1 ng

KEY WORDS

powder; ointment; normal phase; derivatization

REFERENCE

Tsuji, K.; Goetz, J.F.; VanMeter, W.; Gusciora, K.A. Normal-phase high-performance liquid chromatographic determination of neomycin sulfate derivatized with 1-fluoro-2,4-dinitrobenzene. *J.Chromatogr.*, **1979**, *175*, 141-152

SAMPLE

Matrix: milk

Sample preparation: Prepare a SPE column as follows. Shake Amberlite CG 50 resin in buffer, equilibrate for 2 h, fill a plugged Pasteur pipette to a height of 35 mm with the slurry, wash with water until the eluent is neutral. 40 mL Milk + 2 g NaCl, shake well, add a 10 mL aliquot to the SPE column, wash with 8 mL water, add 600 μ L reagent, let stand for 2 min, elute with 3 mL MeOH:buffer 80:20, make up the eluate to 4 mL with MeOH, store at -8 to -10°, after 15 min inject a 10-20 μ L aliquot. (Prepare buffer by dissolving 76 g potassium tetraborate in 400 mL water, adjusting pH to 11 with KOH, and making up to 500 mL with water. Prepare reagent by dissolving 100 mg o-phthalaldehyde in 10 mL MeOH and adding 200 μ L 2-mercaptoethanol and 10 mL borate buffer. Prepare borate buffer by dissolving 3.1 g boric acid in 100 mL water and adjusting the pH to 10.5 with 50% KOH, make up to 125 mL with water.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m HISEP (Supelco)

Mobile phase: Gradient. A was 2 g/L tripotassium EDTA in MeOH:water 70:30. B was MeOH. A:B 100:0 to 40:60 over 15 min (LDC concave curve 4). (This curve holds the initial conditions for about 6 min.)

Flow rate: 1.7

Injection volume: 10-20

Detector: F ex 340 em KV418 filter

CHROMATOGRAM

Retention time: 8.2, 19.8

Limit of detection: 50 ppb

KEY WORDScow; derivatization; SPE

REFERENCE

Agarwal, V.K. High performance liquid chromatographic determination of neomycin in milk using a HISEP column. *J.Liq.Chromatogr.*, **1990**, *13*, 2475-2487

SAMPLE**Matrix:** milk**Sample preparation:** 1 mL Skim milk + 100 μ L 20% trichloroacetic acid, vortex, centrifuge at 4° at 4000 rpm for 30 min. Remove a 180 μ L aliquot and add it to 20 μ L 100 mM sodium 1-pentanesulfonate containing 70 mM acetic acid, vortex, inject a 25 μ L aliquot.

HPLC VARIABLES**Guard column:** 20 \times 4.6 5 μ m Supelguard LC-8-DB**Column:** 150 \times 4.6 5 μ m Supelcosil LC-8-DB**Mobile phase:** MeOH:buffer 1.5:98.5 (Buffer contained 10 mM pentanesulfonic acid, 56 mM sodium sulfate, and 7 mM acetic acid.)**Column temperature:** 32.5**Injection volume:** 25**Detector:** F ex 340 em 455 following post-column reaction. The column effluent mixed with o-phthalaldehyde solution (Pierce) and flowed through a reaction coil at 33° to the detector.

CHROMATOGRAM**Retention time:** 20**Limit of detection:** 150 ng/mL

KEY WORDScow; derivatization; post-column reaction

REFERENCE

Shaikh, B.; Jackson, J. Determination of neomycin in milk by reversed phase ion-pairing liquid chromatography. *J.Liq.Chromatogr.*, **1989**, *12*, 1497-1515

SAMPLE**Matrix:** perilymph**Sample preparation:** Lyophilize 6 μ L perilymph, reconstitute with 90 μ L pyridine, add 10 μ L benzoyl chloride, heat at 80° for 30 min, evaporate to dryness under a stream of nitrogen, add 1 mL MeOH, heat at 80° for 10 min, add 50 mg solid sodium carbonate, add 1 mL MeOH saturated with sodium carbonate, wash 3 times with 2 mL portions of n-hexane, add 1 mL water, remove any hexane which separates, extract with 3 mL chloroform. Wash the chloroform layer 3 times with 1 mL portions of MeOH:water 50:50, evaporate the chloroform layer to dryness, reconstitute with 15 μ L chloroform, inject a 5 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5-6 μ m Zorbax SIL**Mobile phase:** n-Hexane:THF 50:50**Flow rate:** 2**Injection volume:** 5**Detector:** UV 230

CHROMATOGRAM**Retention time:** 8.11, 9.28, 10.08**Limit of detection:** 10 ng

OTHER SUBSTANCES

Simultaneous: dihydrostreptomycin, kanamycin, streptomycin

KEY WORDS

derivatization; normal phase; guinea pig

REFERENCE

Harada, T.; Iwamori, M.; Nagai, Y.; Nomura, Y. Analysis of aminoglycoside antibiotics as benzoyl derivatives by high-performance liquid chromatography and its application to the quantitation of neomycin in the perilymph. *J.Chromatogr.*, **1985**, *337*, 187-193

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-8

Mobile phase: THF:buffer 3:97 (Buffer was 7 mM acetic acid containing 5.6 mM sodium sulfate and 10 mM sodium pentanesulfonate.)

Flow rate: 1.75

Injection volume: 20

Detector: F ex 365 em 418 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 50 \times 4.6 column packed with 75 μ m glass beads and a 3.05 m \times 0.5 mm knitted PTFE coil at 40° to the detector. (The reagent was 400 mM boric acid containing 380 mM KOH, 6 mL/L 40% Brij-35, 4 mL/L mercaptoethanol, and 0.8 g/L orthophthalaldehyde.)

CHROMATOGRAM

Retention time: 5

KEY WORDS

post-column reaction

REFERENCE

Supelco Chromatography Products, Supelco, Inc., Bellefonte PA, 1996, p. A29

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m Ultrasphere octyl

Mobile phase: MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 1.99

Internal standard: naproxen (3.89)

OTHER SUBSTANCES

Simultaneous: bacitracin, cortisone acetate, diazepam, diclofenac, fluorometholone, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, levobunolol, meclofenamic acid, prednisolone acetate, proparacaine, 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

Interfering: metipranolol, propranolol

KEY WORDS

human; rabbit

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: solutions

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 \times 5 Hypersil ODS

Mobile phase: MeOH:water:acetic acid 63.75:31.25:5 containing 5 g/L heptanesulfonic acid

Flow rate: 2

Injection volume: 50

Detector: UV 330

CHROMATOGRAM

Retention time: 21

KEY WORDS

reaction mixtures; derivatization

REFERENCE

Lovering, A.M.; White, L.O.; Reeves, D.S. Identification of aminoglycoside-acetyating enzymes by high-pressure liquid chromatographic determination of their reaction products. *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeCN:water 17:83 containing 16 mM sodium 1-hexanesulfonate and 20 mM Na_3PO_4 , pH 3.5 (Connect a 250 \times 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: 1.5

Injection volume: 25

Detector: RI

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES**Simultaneous:** paromomycin

REFERENCE

Whall, T.J. Determination of streptomycin sulfate and dihydrostreptomycin sulfate by high-performance liquid chromatography. *J.Chromatogr.*, **1981**, *219*, 89–100

SAMPLE**Matrix:** tissue

Sample preparation: Homogenize (Tissumizer) 1 g Ground tissue + 4 mL buffer at medium speed for 1 min, centrifuge at 3600 g for 20 min, remove the supernatant, re-homogenize pellet in 4 mL buffer for 10 min, centrifuge. Combine the supernatants, heat in a boiling water bath with occasional mixing for 5 min, centrifuge at 2000 g for 20 min, remove the supernatant, vortex the precipitate with 2 mL buffer for 30 s, centrifuge at 2000 g for 10 min. Combine the supernatants, acidify to pH 3.5-4 with 50-60 μ L sulfuric acid, centrifuge at 2000 g for 10 min, inject an aliquot of the supernatant. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water, pH 8.0.)

HPLC VARIABLES**Guard column:** 10 μ m RP-18**Column:** 150 \times 4.6 5 μ m 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 μ m), 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:** 22**Limit of detection:** 3.5 ng

OTHER SUBSTANCES**Extracted:** paromomycin**Simultaneous:** dihydrostreptomycin, streptomycin

KEY WORDS

kidney; muscle; cow; pig; post-column reaction

REFERENCE

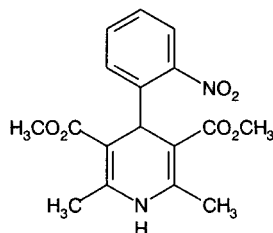
Shaikh, B.; Allen, E.H.; Gridley, J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection. *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36

Nifedipine

Molecular formula: C₁₇H₁₈N₂O₆

Molecular weight: 346.3

CAS Registry No.: 21829-25-4



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)) KH₂PO₄ 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 236

CHROMATOGRAM

Retention time: 4.21

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, albuterol, alimemazine, alminoprofen, alpidem, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cyproheptadine, cytarabine, dacarbazine, daunorubicin, demexiptiline, desipramine, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamine, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephensesin, mepivacaine, metapramine, metformin, methadone, methocarbamol, methotrexate, metipranolol, metoclopramide, mexiletine, mianserine, midazolam, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, niflumic acid, nimodipine, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxprenolol, penbutolol, penfluridol, phencyclidine, phenobarbital, phenol, phenylbutazone,

pimozide, pindolol, pipamperone, prazepam, prazosin, procainide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfipyrazole, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, ticlopidine, timolol, tiocloamarol, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, zolpidem, zorubicine

Interfering: ajmaline, alprazolam, celiprolol, cycloguanil, debrisoquine, dextromethorphan, disopyramide, ketamine, lorazepam, mephentermine, methaqualone, metoprolol, minoxidil, nitrazepam, oxazepam, pentazocine, piroxicam, prilocaine, quinidine, quinine, sulindac, tiaprofenic acid, tofisopam, yohimbine, zopiclone

KEY WORDS

whole blood; plasma

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 + 50 μ L 10 μ g/mL nisoldipine in MeOH + 100 μ L 1 M NaOH, vortex for 3 s, add 5 mL 75:25 MTBE:isooctane, vortex for 30 s, centrifuge at 1800 g for 5 min. Transfer upper layer to a 100 \times 13 mm glass tube, evaporate to dryness without heating using a Speed Vac concentrator evaporator. Reconstitute in 200 μ L mobile phase, vortex for 15 s, inject a 150 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 8 μ m C8 Nova-Pak radial pack

Mobile phase: MeOH:water 65:35 adjusted to approx. pH 4 with 1% acetic acid and 0.03% triethylamine

Flow rate: 1.1

Injection volume: 150

Detector: UV 350

CHROMATOGRAM

Retention time: 6.5

Internal standard: nisoldipine (16)

Limit of detection: 1 ng/mL

Limit of quantitation: 5 ng/mL

KEY WORDS

plasma; analysis conducted under sodium lamps

REFERENCE

Grundy, J.S.; Kherani, R.; Foster, R.T. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. *J. Chromatogr. B*, **1994**, *654*, 146–151

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 150 ng nitrazepam + 100 μ L 1 M NaOH + 5 mL hexane:dichloromethane 70:30, shake horizontally for 20 min, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 120 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm RP-18 (Kontron)

Mobile phase: MeOH:water:glacial acetic acid 70:30:1

Flow rate: 1.5

Injection volume: 20

Detector: UV 238

CHROMATOGRAM

Retention time: 4.2

Internal standard: nitrazepam (3.8)

Limit of detection: 5 ng/mL

Limit of quantitation: 10 ng/mL

KEY WORDS

serum; protect from light; pharmacokinetics

REFERENCE

Jankowski, A.; Lamparczyk, H. Evaluation of chromatographic methods for the determination of nifedipine in human serum. *J.Chromatogr.A*, **1994**, 668, 469–473

SAMPLE

Matrix: blood

Sample preparation: Centrifuge plasma, inject a 650 μL aliquot onto column A and elute to waste with mobile phase A, after 45 s inject 650 μL MeOH:water 20:80, continue to elute column A to waste with mobile phase A, after 45 s 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 for at least 45 s.

HPLC VARIABLES

Column: A 10 × 4 25-40 μm LiChroprep RP-2; B 125 × 4 5 μm Hypersil ODS

Mobile phase: A 20 mM Ammonium carbamate; B MeCN:20 mM pH 4.0 acetate buffer 50:50

Flow rate: A 3; B 1

Injection volume: 650

Detector: UV 355

CHROMATOGRAM

Retention time: 2.4

Limit of detection: 0.5 ng/mL

KEY WORDS

plasma; pharmacokinetics; column-switching; protect from light

REFERENCE

Nitsche, V.; Schütz, H.; Eichinger, A. Rapid high-performance liquid chromatographic determination of nifedipine in plasma with on-line precolumn solid-phase extraction. *J.Chromatogr.*, **1987**, 420, 207–211

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 50 μL 4 μg/mL nisoldipine in mobile phase, adjust to pH 9 with 1 M KOH, add 0.5 g KCl, add 3 mL diethyl ether, shake gently (Fisher Roto-Rack) for 10 min, centrifuge at 1000 rpm for 15 min. Separate and retain ether layer, extract aqueous layer as before. Evaporate all ether layers to dryness under dry nitrogen

at 20°. Reconstitute the residue in 50 μ L mobile phase, inject a 20 μ L aliquot. (Wrap tubes in Al foil.)

HPLC VARIABLES

Column: 250 \times 4 Brownlee 10 μ m RP8

Mobile phase: MeOH:50 mM buffer 30:70 (Buffer was 12 g NaH₂PO₄ in 2 L water adjusted to pH 4 \pm 0.5 with 80% phosphoric acid.)

Flow rate: 0.75

Injection volume: 20

Detector: UV 350

CHROMATOGRAM

Retention time: 7

Internal standard: nisoldipine (13)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: photodegradation products of nifedipine, furosemide, hydrochlorothiazide, timolol, triamterene

Noninterfering: aspirin, acetaminophen, caffeine, chlorthalidone, diazepam, methyldopa, oxprenolol, propranolol

KEY WORDS

serum

REFERENCE

Snedden, W.; Fernandez, P.G.; Galway, B.A.; Kim, B.K. Specific HPLC assay for serum nifedipine. *Clin. Invest. Med.*, **1984**, 7, 173-178

SAMPLE

Matrix: perfusate

Sample preparation: Add perfusate to an equal volume of nitrendipine in MeOH, vortex, centrifuge, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Nucleosil 5C18

Mobile phase: MeCN:water 40:60

Detector: UV 235

CHROMATOGRAM

Internal standard: nitrendipine

KEY WORDS

protect from light

REFERENCE

Kobayashi, D.; Matsuzawa, T.; Sugibayashi, K.; Morimoto, Y.; Kobayashi, M.; Kimura, M. Feasibility of use of several cardiovascular agents in transdermal therapeutic systems with *l*-menthol-ethanol system on hairless rat and human skin. *Biol. Pharm. Bull.*, **1993**, 16, 254-258

SAMPLE

Matrix: solutions

Sample preparation: Inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 33 \times 4.6 3 μ m Supelcosil LC-18-DB

Mobile phase: Gradient. MeCN:buffer from 10:90 to 50:50 over 10 min, maintain at 50:50 for 2 min. (Buffer was 25 mM KH_2PO_4 containing 0.02% triethylamine, pH 3.0.)

Column temperature: 25

Flow rate: 2

Injection volume: 5

Detector: UV 220

CHROMATOGRAM

Retention time: 8.2

OTHER SUBSTANCES

Simultaneous: digoxin, diltiazem, dipyridamole, dobutamine, flunarizine, lidoflazine, oxiprenolol, pindolol, verapamil

REFERENCE

Supelco Chromatography Products, Supelco, Inc., Bellefonte PA, 1996, p. A27

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: 7.42 (A), 7.91 (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, fenpropfen, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 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, bromazepam, 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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylprylon, meto-

prolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 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: 3.24

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, diltiazem, metoprolol, 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

Sample preparation: Centrifuge at 2000 g at 37° for 15 min, inject an aliquot.

HPLC VARIABLES

Column: 100 × 8 5 μm C18 Novapak

Mobile phase: MeCN:10 mM pH 4.5 phosphate buffer 70:30

Flow rate: 2

Detector: UV 237

OTHER SUBSTANCES

Also analyzed: felodipine, nicardipine, nimodipine, nitrendipine

KEY WORDS

buffers

REFERENCE

Diez, I.; Colom, H.; Moreno, J.; Obach, R.; Peraire, C.; Domenech, J. A comparative in vitro study of transdermal absorption of a series of calcium channel antagonists. *J.Pharm.Sci.*, **1991**, *80*, 931-934

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 10 μ L aliquot of a solution in MeCN.

HPLC VARIABLES**Column:** 100 \times 4.6 CS-MP Spheri-5 cyano**Mobile phase:** Gradient. MeCN:buffer from 10:90 to 40:60 over 10 min, re-equilibrate for 5 min. (Buffer was 50 mM KH_2PO_4 adjusted to pH 3 with phosphoric acid.)**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV 200; UV 272; UV 276; UV 280; UV 314

CHROMATOGRAM**Retention time:** 8.5

OTHER SUBSTANCES**Simultaneous:** degradation products, nitrendipine

REFERENCE

Logan, B.K.; Patrick, K.S. Photodegradation of nifedipine relative to nitrendipine evaluated by liquid and gas chromatography. *J.Chromatogr.*, **1990**, *529*, 175-181

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 125 \times 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, bamethane, 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, di-

methothiazine, 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, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazolin, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilone, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylephedrine, methylephedrine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, 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, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimetho-prim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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- Hayase, N.; Itagaki, Y.-I.; Ogawa, S.; Akutsu, S.; Inagaki, S.-I.; Abiko, Y. Newly discovered photodegradation products of nifedipine in hospital prescriptions. *J.Pharm.Sci.*, **1994**, *83*, 532–538 [simultaneous degradation products; column temp 30; tablets]
- Kobayashi, D.; Matsuzawa, T.; Sugibayashi, K.; Morimoto, Y.; Kimura, M. Analysis of the combined effect of l-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. *Pharm.Res.*, **1994**, *11*, 96–103 [also atenolol, morphine, naloxone, nitrendipine, vinpocetine]
- Thongnopnua, P.; Viwatongsa, K. Quantitative analysis of nifedipine in plasma by high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 119–125 [LOQ 7 ng/mL; butamben (IS)]
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- Ohkubo, T.; Noro, H.; Sugawara, K. High-performance liquid chromatographic determination of nifedipine and a trace photodegradation product in hospital prescriptions. *J.Pharm.Biomed.Anal.*, **1992**, *10*, 67–70
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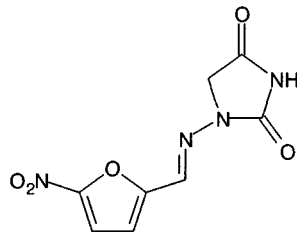
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- Pietta, P.; Rava, A.; Biondi, P. High-performance liquid chromatography of nifedipine, its metabolites and photochemical degradation products. *J.Chromatogr.*, **1981**, *210*, 516–521

Nitrofurantoin

Molecular formula: C₈H₆N₄O₅

Molecular weight: 238.2

CAS Registry No.: 67-20-9, 54-87-5 (sodium salt), 17140-81-7 (monohydrate)



SAMPLE

Matrix: blood, milk

Sample preparation: 1 mL Plasma or milk + furazolidone, extract with 5 mL dichloromethane in acidic medium (pH 3), mix, centrifuge. Remove organic phase and evaporate it to dryness at 50° under a stream of nitrogen. Take up residue in MeCN and inject an aliquot. (Protect from light during extraction procedure.)

HPLC VARIABLES

Column: 75 × 4.6 Beckman XL 3 μm ODS

Mobile phase: MeCN:water 35:65

Flow rate: 1

Detector: UV 364

CHROMATOGRAM

Internal standard: furazolidone

Limit of detection: 10 ng/mL

KEY WORDS

plasma

REFERENCE

Pons, G.; Rey, E.; Richard, M.O.; Vauzelle, F.; Francoual, C.; Moran, C.; d'Athis, P.; Badoual, J.; Olive, G. Nitrofurantoin excretion in human milk. *Dev.Pharmacol.Ther.*, **1990**, *14*, 148–152

SAMPLE

Matrix: cell suspensions

Sample preparation: 200 μL Cell suspension + 200 μL 10% trichloroacetic acid + 100 μL 10 μg/mL furazolidone, centrifuge, inject a 75 μL aliquot of the supernatant.

HPLC VARIABLES

Column: C18 (Waters)

Mobile phase: MeOH:water:glacial acetic acid 20:80:0.09, adjusted to pH 5 with NaOH

Flow rate: 1.5

Injection volume: 75

Detector: UV 370

CHROMATOGRAM

Retention time: 6

Internal standard: furazolidone (8)

REFERENCE

Minchin, R.F.; Ho, P.C.; Boyd, M.R. Reductive metabolism of nitrofurantoin by rat lung and liver in vitro. *Biochem.Pharmacol.*, **1986**, *35*, 575–580

SAMPLE

Matrix: eggs, tissue

Sample preparation: Blend (Stomacher) 10 g homogenized tissue with 30 mL saline for 3 min, centrifuge at 2000 g, mix 20 mL of the supernatant with 2 mL 1% sodium azide. Dilute 10 mL homogenized egg with 10 mL saline, add 3 mL 10% sodium azide solution. Dialyze sample using a Cuprophane membrane (10000-15000 dalton cut-off) against water pumped at 0.36-1.44 mL/min for 3-9 min, pass the water through column A, flush the column with pure water for 8 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. To increase sensitivity a number of sample batches can be dialyzed before the contents of column A are analyzed. (Caution! Sodium azide is carcinogenic, mutagenic, and highly toxic! Do not discharge to the sink!)

HPLC VARIABLES

Column: A 60 × 4.6 37-50 μm Bondapak C18/Corasil; B 250 × 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:100 mM pH 5 acetate buffer 20:80

Flow rate: 1

Detector: UV 365

CHROMATOGRAM

Retention time: 8

Limit of detection: 1 ng/g (eggs); 2 ng/g (tissue)

OTHER SUBSTANCES

Extracted: furaltadone, furazolidone, nitrofurazone

KEY WORDS

protect from light; cow; muscle; dialysis

REFERENCE

Aerts, M.M.; Beek, W.M.; Brinkman, U.A. On-line combination of dialysis and column-switching liquid chromatography as a fully automated sample preparation technique for biological samples. Determination of nitrofurantoin residues in edible products. *J.Chromatogr.*, **1990**, *500*, 453-468

SAMPLE

Matrix: feed, formulations, milk

Sample preparation: Formulations. Dissolve formulation in DMF, filter, inject a 10 μL aliquot. Feeds. Stir 10 g finely ground feeds with 40 mL DMF for 30 min, centrifuge, filter, wash residues with DMF, dilute to 50 mL with DMF, inject a 10 μL aliquot. Milk. Lyophilize 200 mL milk, wash with 75 mL MeCN during 15 min. Extract residue with 15 mL DMF with stirring for 30 min, wash residue with a mixture of 25 mL MeCN + 5 mL DMF. Combine all organic solutions and evaporate to dryness in vacuum. Treat residue with DMF, filter, dilute to 25 mL with DMF, filter before analysis, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 33 × 4.6 Perkin-Elmer Pecosphere 3x3 CR C18

Mobile phase: MeCN:100 mM pH 3.2 sodium acetate/acetic acid 10:90

Flow rate: 2

Injection volume: 10

Detector: UV 360

CHROMATOGRAM

Retention time: 1.36

Limit of detection: 4.7 ng

OTHER SUBSTANCES

Simultaneous: furaltadone, furazolidone

REFERENCE

Galeano Díaz, T.; Lopez Martínez, L.; Martínez Galera, M.; Salinas, F. Rapid determination of nitrofurantoin, furazolidone and furaltadone in formulations, feed and milk by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1994**, *17*, 457–475

SAMPLE

Matrix: formulations

Sample preparation: Dissolve tablet in 10 mM HCl containing 90 mM KCl (pH 2.0), inject an aliquot.

HPLC VARIABLES

Column: 50 mm long ODS Hypersil C18

Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 30:70

Flow rate: 1

Detector: UV 257

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Simultaneous: hydrocortisone

KEY WORDS

tablets

REFERENCE

Neervannan, S.; Dias, L.S.; Southard, M.Z.; Stella, V.J. A convective-diffusion model for dissolution of two non-interacting drug mixtures from co-compressed slabs under laminar hydrodynamic conditions. *Pharm.Res.*, **1994**, *11*, 1288–1295

SAMPLE

Matrix: formulations

Sample preparation: 5 mL Suspension + 20 mL water + 50 mL DMF, shake for 10 min, cool to room temperature, dilute to 100 mL with DMF, centrifuge an aliquot. 4 mL Supernatant + 15 mL 65 µg/mL acetanilide in mobile phase, filter (5 µm), discard first 2 mL, inject a 15 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeCN:buffer 12:88 (Buffer was 6.8 g KH₂PO₄ in 500 mL water, add 30 mL 1 M NaOH, dilute to 1 L with water, pH was 7.0.)

Flow rate: 1.5

Injection volume: 15

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Internal standard: acetanilide

KEY WORDS

suspensions

REFERENCE

Juenge, E.C.; Kreienbaum, M.A.; Gurka, D.F. Assay of nitrofurantoin oral suspensions contaminated with 3-(5-nitrofururylideneamino)hydantoic acid. *J.Pharm.Sci.*, **1985**, *74*, 100–102

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: 9.8

Limit of detection: 22 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, carbadox, furaltadone, furazolidone, nitrofurazone

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 (Tekmar Tissuemizer SDT 1810 with SDT 182 EN shaft/blades) 5 g finely chopped muscle tissue and 20 mL MeCN at medium speed for 45 s, centrifuge at 1500 g for 5 min, add 15 mL MeCN-saturated hexane, shake for 30 s, discard the hexane layer, repeat the wash. Add 9 mL EtOH to the MeCN layer and evaporate under reduced pressure to 2-5 mL at 45°, add 2 mL EtOH, evaporate to 2 mL, add 2 mL EtOH, evaporate to dryness, add 1 mL mobile phase, sonicate for 5 min, centrifuge at 15400 g for 10 min, filter (0.45 µm) the supernatant, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 200 × 4.6 µm ODS Hypersil C18

Mobile phase: MeCN:1% acetic acid 25:75

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: UV 375

CHROMATOGRAM

Retention time: 3.5

Limit of detection: 1 ng/g

Limit of quantitation: 5 ng/g

OTHER SUBSTANCES

Extracted: metabolites, furazolidone, nitrofurazone

KEY WORDS

fish; muscle

REFERENCE

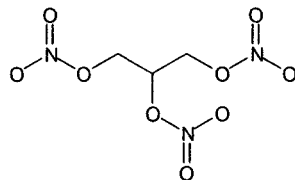
Rupp, H.S.; Munns, R.K.; Long, A.R.; Plakas, S.M. Simultaneous determination of nitrofurazone, nitrofurantoin, and furazolidone in channel catfish (*Ictalurus punctatus*) muscle tissue by liquid chromatography. *J.AOAC Int.*, **1994**, *77*, 344–350

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Ebel, S.; Liedtke, R.; Missler, B. [Quantitative determination of nitrofurantoin in body fluids by direct injection HPLC]. *Arch.Pharm.(Weinheim)*, **1980**, *313*, 95–96

Jonen, H.G.; Oesch, F.; Platt, K.L. 4-Hydroxylation of nitrofurantoin in the rat. A 3-methylcholanthrene-inducible pathway of a relatively nontoxic compound. *Drug Metab.Dispos.*, **1980**, *8*, 446–451

Nitroglycerin



Molecular formula: C₃H₅N₃O₉

Molecular weight: 227.1

CAS Registry No.: 55-63-0

SAMPLE

Matrix: blood

Sample preparation: 3 mL Plasma + 12 mL dichloromethane:ethyl acetate 1:1, shake mechanically at 250 cycles/min for 5 min, centrifuge at 550 g at 4° for 5 min. Remove the organic phase and evaporate it to about 20 µL under a stream of nitrogen at room temperature, inject.

HPLC VARIABLES

Column: 250 × 4 10 µm Zorbax NH₂

Mobile phase: n-Hexane:MeOH 95:5

Flow rate: 5

Injection volume: 20

Detector: Thermal energy analyzer, Thermo Electron Corp. Model 502A, furnace temp 575°, argon 15 mL/min, oxygen 25 mL/min, MeOH/dry ice slush bath

CHROMATOGRAM

Retention time: 5.0

Internal standard: nitroglycerin

OTHER SUBSTANCES

Extracted: isosorbide dinitrate, isosorbide mononitrate

KEY WORDS

plasma; nitroglycerin is IS

REFERENCE

Maddock, J.; Lewis, P.A.; Woodward, A.; Massey, P.R.; Kennedy, S. Determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by high-performance liquid chromatography-thermal energy analysis. *J.Chromatogr.*, **1983**, 272, 129-136

SAMPLE

Matrix: formulations

Sample preparation: Inject directly.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeCN:MeOH:water 6:6:13

Column temperature: 35

Flow rate: 2.8

Detector: UV 210

CHROMATOGRAM

Retention time: 2.8

KEY WORDS

injections; stability-indicating

REFERENCE

Driver, P.S.; Jarvi, E.J.; Gratzner, P.L. Stability of nitroglycerin as nitroglycerin concentrate for injection stored in plastic syringes. *Am.J.Hosp.Pharm.*, **1993**, *50*, 2561–2563

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: C18

Mobile phase: MeOH:water 50:50

Flow rate: 1.3

Detector: UV 220

CHROMATOGRAM

Internal standard: isosorbide dinitrate

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 saline, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Lichrosorb 10 RP 8

Mobile phase: MeOH:water 50:50

Flow rate: 2

Injection volume: 10

Detector: UV 214

CHROMATOGRAM

Retention time: 5.8

OTHER SUBSTANCES

Simultaneous: isosorbide dinitrate

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: formulations

Sample preparation: Weigh out an amount of finely powdered tablets or capsules equivalent to about 25 mg of drug. Add 50 mL buffer, shake for 30 min, add 10 mL MeOH, make up to 100 mL with buffer, filter (0.45 μ m), inject a 20 μ L aliquot. If the sample clumps when the buffer is added, agitate with a stirring rod and sonicate. (Buffer was MeOH:200 mM pH 4.7 ammonium acetate buffer:water 55:10:35.)

HPLC VARIABLES

Guard column: 50 × 6.4 25-37 μm Whatman Co-Pell ODS

Column: 250 × 4.6 5 μm Ultrasphere ODS

Mobile phase: MeOH:200 mM pH 4.7 ammonium acetate buffer: water 55:10:35

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Internal standard: nitroglycerin

OTHER SUBSTANCES

Simultaneous: isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate, saccharin

KEY WORDS

tablets; capsules; nitroglycerin is IS

REFERENCE

Carlson, M.; Thompson, R.D.; Snell, R.P. Determination of isosorbide dinitrate in pharmaceutical products by HPLC. *J.Chromatogr.Sci.*, **1988**, *26*, 574-578

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 150 × 4.6 5 μm C8 (Altex)

Mobile phase: MeCN:water 40:60

Flow rate: 2.67

KEY WORDS

tablets; mouth rinses

REFERENCE

Noonan, P.K.; Benet, L.Z. Incomplete and delayed bioavailability of sublingual nitroglycerin. *Am.J.Cardiol.*, **1985**, *55*, 184-187

SAMPLE

Matrix: formulations

Sample preparation: 1 mL Solution + 300 μL isosorbide dinitrate solution, mix, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl

Mobile phase: MeCN:THF:water 26:10:64

Flow rate: 2

Detector: UV 218

CHROMATOGRAM

Internal standard: isosorbide dinitrate

KEY WORDS

injections; 5% dextrose; saline

REFERENCE

Thompson, M.; Smith, M.; Gragg, R.; Soliman, K.F. Stability of nitroglycerin and dobutamine in 5% dextrose and 0.9% sodium chloride injection. *Am.J.Hosp.Pharm.*, **1985**, *42*, 361-362

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, weigh out a portion equivalent to 3 mg erythrityl tetranitrate, add to 10 mL 75 μ g/mL nitroglycerin in MeOH, sonicate for 2 min, shake mechanically for 30 min, filter, inject an aliquot

HPLC VARIABLES

Guard column: 40 \times 4.6 μ Bondapak C18/Corasil

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:water 40:60

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 14

Internal standard: nitroglycerin

OTHER SUBSTANCES

Simultaneous: erythrityl tetranitrate, isosorbide dinitrate, pentaerythritol tetranitrate

KEY WORDS

tablets; nitroglycerin is IS

REFERENCE

Olsen, C.S.; Scroggins, H.S. High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in various tablet forms. *J.Pharm.Sci.*, **1984**, *73*, 1303-1304

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak phenyl

Mobile phase: MeCN:THF:water 26:10:64

Flow rate: 2

Injection volume: 10-40

Detector: UV 218

CHROMATOGRAM

Retention time: 10

Internal standard: isosorbide dinitrate (6)

KEY WORDS

injections; stability-indicating

REFERENCE

Baaske, D.M.; Carter, J.E.; Amann, A.H. Rapid and accurate stability-indicating assay for nitroglycerin. *J.Pharm.Sci.*, **1979**, *68*, 481-483

SAMPLE

Matrix: solutions

Sample preparation: Add 10 g 10% nitroglycerin solution in lactose to 125 mL MeOH, sonicate for 5 min, shake mechanically for 30 min, dilute to 200 mL with MeOH, let undissolved lactose settle, filter through paper. Dilute 5 mL to 250 mL with mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere ODS C18

Mobile phase: MeCN:water 65:35

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 6

Internal standard: nitroglycerin

OTHER SUBSTANCES

Simultaneous: pentaerythritol tetranitrate

KEY WORDS

collaborative study; nitroglycerin is IS

REFERENCE

Carlson, M. Liquid chromatographic determination of pentaerythritol tetranitrate in pharmaceuticals: collaborative study. *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 693-697

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-8

Mobile phase: MeOH:water 50:50

Flow rate: 1.5

Detector: UV 210

CHROMATOGRAM

Retention time: 4.5-5

REFERENCE

Roberts, M.E.; Mueller, K.R. Comparisons of in vitro nitroglycerin (TNG) flux across yucatan pig, hairless mouse, and human skins. *Pharm.Res.*, **1990**, *7*, 673-676

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 33 \times 4.6 3 μ m C18 (Perkin-Elmer)

Mobile phase: MeCN:MeOH:water 24:24:52

Flow rate: 3.5

Detector: UV 210

CHROMATOGRAM**Retention time:** 0.6**Limit of quantitation:** 1 µg/mL

OTHER SUBSTANCES**Simultaneous:** degradation products

REFERENCE

Severin, G. Rapid high-performance liquid chromatographic procedure for nitroglycerin and its degradation products. *J.Chromatogr.*, **1985**, *320*, 445–449

ANNOTATED BIBLIOGRAPHY

Carlson, M.; Thompson, R.D. Determination of pentaerythritol tetranitrate in pharmaceuticals by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1988**, *11*, 2603–2620 [simultaneous pentaerythritol tetranitrate; tablets; capsules; nitroglycerin is IS]

Torok, I.; Paal, T.; Koszegine Szalai, H.; Keseru, P. [High performance liquid chromatographic assay and determination of the even distribution of active ingredients in nitroglycerin and isosorbide dinitrate tablets]. *Acta Pharm.Hung.*, **1985**, *55*, 154–162

Baaske, D.M.; Karnatz, N.N.; Carter, J.E. High-performance liquid chromatographic assay for partially nitrated glycerins in nitroglycerin. *J.Pharm.Sci.*, **1983**, *72*, 194–196

Gelber, L.; Papas, A.N. Validation of high-performance liquid chromatographic methods for analysis of sustained-release preparations containing nitroglycerin, isosorbide dinitrate, or pentaerythritol tetranitrate. *J.Pharm.Sci.*, **1983**, *72*, 124–126

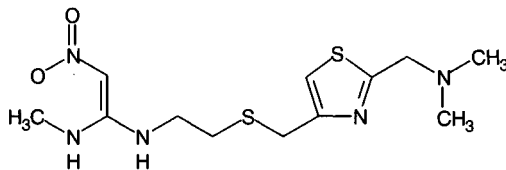
Yu, W.C.; Goff, E.U. Determination of vasodilators and their metabolites in plasma by liquid chromatography with a nitrosyl-specific detector. *Anal.Chem.*, **1983**, *55*, 29–32 [TEA detection; plasma; SPE; extracted isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate; LOD 0.1 ng]

Nizatidine

Molecular formula: C₁₂H₂₁N₅O₂S₂

Molecular weight: 331.5

CAS Registry No.: 76963-41-2



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)) KH₂PO₄ 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 322

CHROMATOGRAM

Retention time: 3.52

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzoylegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nizatidine, nitraxepam, nitrendipine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine,

procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, secobarbital, strychnine, sulfinpyrazole, sulindac, sulpride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: amisulpride, benzocaine, carteolol, codeine, lisinopril, mephenesin, nalorphine, naloxone, naltrexone, nizatidine, ritodrine, sotalol, sultopride

KEY WORDS

whole blood; plasma

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 or saliva. 1 mL Plasma or saliva + 200 ng IS + 250 μ L saturated sodium carbonate + 500 μ L saturated solution of sodium sulfate in EtOH, extract into 5 mL dichloromethane. Evaporate the organic layer and dissolve the residue in 200 μ L 100 mM HCl:mobile phase 1:1, inject an aliquot. Urine. 1 mL Urine + 2000 ng IS + 1 mL saturated sodium carbonate, extract into 5 mL dichloromethane. Evaporate the organic layer and dissolve the residue in 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Brownlee Spheri-10 RP-8 guard column

Column: 250 \times 4.6 μ m Zorbax C8

Mobile phase: MeCN containing 1% triethylamine:20 mM ammonium acetate 18.5:81.5

Flow rate: 1

Detector: UV 313

CHROMATOGRAM

Internal standard: N1-ethylnizatidine

Limit of detection: 10 ng/mL

KEY WORDS

plasma

REFERENCE

Knadler, M.P.; Bergstrom, R.F.; Callaghan, J.T.; Rubin, A. Nizatidine, an H₂-blocker. Its metabolism and disposition in man. *Drug Metab. Dispos.*, 1986, 14, 175-182

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 25 μ L Plasma + 100 μ L water + 100 μ L 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 1650 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot. (Hemolyze 25 μ L whole blood with 200 μ L water then proceed as above.) Tissue. Homogenize brain tissue with 100 μ L water and 1 mL saline at 0° for 1 min, add 100 μ L 500 mM NaOH, extract with 5 mL dichloromethane. Remove 3 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L mobile phase, centrifuge at 10000 g, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 × 4 Senshu gel 7C18H (Senshu, Tokyo)

Mobile phase: MeCN:buffer 5:95. (Buffer was 5 mM NaH₂PO₄ containing 5 mM tetramethylammonium chloride.)

Column temperature: 30

Flow rate: 2

Injection volume: 50

Detector: UV 320

CHROMATOGRAM

Internal standard: nizatidine

OTHER SUBSTANCES

Extracted: ranitidine

KEY WORDS

nizatidine is IS; plasma; mouse; brain; whole blood

REFERENCE

Shimokawa, M.; Yamamoto, K.; Kawakami, J.; Sawada, Y.; Iga, T. Effect of renal or hepatic dysfunction on neurotoxic convulsion induced by ranitidine in mice. *Pharm.Res.*, **1994**, *11*, 1519–1523

SAMPLE

Matrix: formulations

Sample preparation: Dilute a 1 mL aliquot to a theoretical concentration of 30 µg/mL with MeCN:water 2:50, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 mm long 5 µm Waters Resolve C18

Mobile phase: MeOH:100 mM ammonium acetate:diethylamine 1280:2720:4, adjusted to a pH of 7.5

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 6.7

OTHER SUBSTANCES

Simultaneous: nizatidine amide, nizatidine sulfoxide, phenol

KEY WORDS

injections; stability-indicating

REFERENCE

Raineri, D.L.; Cwik, M.J.; Rodvold, K.A.; Deyo, K.L.; Scaros, L.P.; Fischer, J.H. Stability of nizatidine in commonly used intravenous fluids and containers. *Am.J.Hosp.Pharm.*, **1988**, *45*, 1523–1529

SAMPLE

Matrix: perfusate, urine

Sample preparation: Urine. Take 10 µL urine diluted 10 times with 10 mM pH 7.5 Na₂HPO₄ buffer, make up volume to 300 µL with 10 mM pH 7.5 Na₂HPO₄ buffer. Place solution on YM-10 ultrafiltration membrane with a cut-off of 10000, centrifuge at 4000 g for 20 min. Mix 180 µL filtrate with 20 µL MeOH, inject 50 µL. Perfusate. Take 10-100

μL perfusate, make up volume to 300 μL with 10 mM pH 7.5 Na_2HPO_4 buffer. Place solution on YM-10 ultrafiltration membrane with a cut-off of 10000, centrifuge at 4000 g for 20 min. Mix 180 μL filtrate with 20 μL MeOH, inject 50 μL .

HPLC VARIABLES

Guard column: 75 \times 2.1 5 μm LiChrosorb RP-18

Column: 150 \times 4.6 5 μm LiChrosorb RP-18

Mobile phase: MeOH: 10 mM pH 7.5 Na_2HPO_4 30:70

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: UV 228

CHROMATOGRAM

Retention time: 10

Internal standard: nizatidine

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: cimetidine

KEY WORDS

nizatidine is IS

REFERENCE

Boom, S.P.A.; Moons, M.M.; Russel, F.G.M. Renal tubular transport of cimetidine in the isolated perfused kidney of the rat. *Drug Metab. Dispos.*, **1994**, 22, 148–153

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 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' 0.43

OTHER SUBSTANCES

Also analyzed: acebutolol, 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, oxprenolol, pheniramine, phentolamine, pindolol, pizotiline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleppamine, 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: 5.57 (A), 3.13 (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, fenopropfen, 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, methylgluta, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, 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, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some details of plasma extraction

REFERENCE

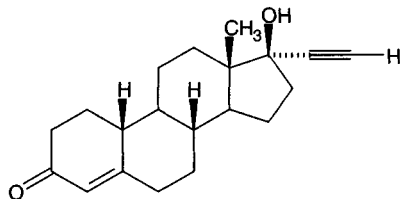
Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology. *J.Chromatogr.A*, **1995**, *692*, 103–119

Norethindrone

Molecular formula: C₂₀H₂₆O₂

Molecular weight: 298.4

CAS Registry No.: 68-22-4, 51-98-9 (acetate)



SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500 μ L plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500 μ L aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L MeOH:water 20:80, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 1.5 μ m Hypersil ODS

Mobile phase: MeCN:MeOH:water 25:25:50

Flow rate: 0.1

Injection volume: 20

Detector: UV

CHROMATOGRAM

Retention time: 20

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: androstenedione, 20 α -hydroxy-4-pregnen-3-one, 17 α -hydroxyprogesterone, progesterone, testosterone

KEY WORDS

microbore; rat; plasma; SPE

REFERENCE

Taylor, R.B.; Kendle, K.E.; Reid, R.G.; Hung, C.T. Chromatography of progesterone and its major metabolites in rat plasma using microbore high-performance liquid chromatography columns with conventional injection and detection systems. *J.Chromatogr.*, **1987**, 385, 383–392

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 5 mL hexane:dichloromethane 1:1, shake for 10 min, centrifuge at 2000 rpm for 10 min. Remove 4 mL of the organic phase and evaporate it at 55° under nitrogen. Reconstitute residue in 300 μ L mobile phase, inject a 150 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m LiChrosorb RP-8

Mobile phase: MeOH:MeCN:water 20:30:50

Flow rate: 2

Injection volume: 150

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Limit of quantitation: 2 ng/mL

KEY WORDS

plasma

REFERENCE

Loo, J.C.K.; Brien, R. Analysis of norethindrone in plasma by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1981**, *4*, 871-877

SAMPLE

Matrix: bulk

HPLC VARIABLES

Column: 250 × 4 10 μm LiChrosorb RP-18

Mobile phase: MeOH:water 70:30

Flow rate: 1

Injection volume: 25

Detector: UV 240

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol, impurities, norgestrel

REFERENCE

Görög, S.; Herényi, B. Analysis of steroids. XXXVIII. The use of high-performance liquid chromatography with diode-array UV detection for estimating impurity profiles of steroid drugs. *J.Chromatogr.*, **1987**, *400*, 177-186

SAMPLE

Matrix: food

Sample preparation: Dissolve apple extracts in THF, inject a 10 μL extract.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:THF:water 11:26:63

Flow rate: 1.7

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 11.6

OTHER SUBSTANCES

Simultaneous: degradation products, impurities

KEY WORDS

apples; fruit; stability-indicating

REFERENCE

Mayberry, D.O.; Kowblansky, M.; Lane, P.A.; Wray, P.E. Determination of norethindrone stability on Red Delicious apples. *J.Pharm.Sci.*, **1990**, *79*, 746-749

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 12 tablets in 600 mL water with stirring at 75 rpm, remove 3 mL sample, centrifuge at 3000 rpm for 15 min, inject a 250 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Phenomenex IB-Sil 3 C18

Mobile phase: MeCN:water 40:60, pH 5.6

Flow rate: 1.2

Injection volume: 250

Detector: UV 200

CHROMATOGRAM

Retention time: 7.7

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol

KEY WORDS

tablets; modification of USP method

REFERENCE

Dorantes, A.; Stavchansky, S. Modification of the U.S.P. dissolution method for the analysis of norethindrone and ethinyl estradiol tablets. *J.Pharm.Sci.*, **1994**, *83*, 379-381

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 6 tablets in 600 mL water:isopropanol 97:3, remove 5 mL samples, centrifuge at 1500 rpm for 10 min, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m Bondapak C18

Mobile phase: MeCN:water 55:45

Flow rate: 1

Injection volume: 50-200

Detector: UV 200

OTHER SUBSTANCES

Simultaneous: mestranol

KEY WORDS

tablets; modified USP method

REFERENCE

Nguyen, H.T.; Shiu, G.K.; Worsley, W.N.; Skelly, J.P. Dissolution testing of norethindrone:ethinyl estradiol, norethindrone:mestranol, and norethindrone acetate:ethinyl estradiol combination tablets. *J.Pharm.Sci.*, **1990**, *79*, 163-167

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 6 tablets in 600 mL 100 mM HCl containing 0.02% sodium lauryl sulfate, remove 5 mL samples, centrifuge at 1500 rpm for 10 min, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Column: 220 \times 4.6 5 μ m Spheri-5 C18

Mobile phase: MeCN:20 mM pH 6.0 phosphate buffer 35:65

Flow rate: 1.5
Injection volume: 50-200
Detector: UV 200

CHROMATOGRAM

Retention time: 20.26

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol

KEY WORDS

tablets; modified USP method

REFERENCE

Nguyen, H.T.; Shiu, G.K.; Worsley, W.N.; Skelly, J.P. Dissolution testing of norethindrone: ethinyl estradiol, norethindrone:mestranol, and norethindrone acetate:ethinyl estradiol combination tablets. *J.Pharm.Sci.*, **1990**, *79*, 163-167

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 6 tablets in 600 mL 100 mM HCl containing 0.02% sodium lauryl sulfate, remove 5 mL samples, centrifuge at 1500 rpm for 10 min, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Column: 220 \times 4.6 5 μ m Spheri-5 C18

Mobile phase: MeCN:20 mM pH 6.0 phosphate buffer 60:40

Flow rate: 1

Injection volume: 50-200

Detector: UV 240

CHROMATOGRAM

Retention time: 12.73 (norethindrone acetate)

KEY WORDS

tablets; modified USP procedure

REFERENCE

Nguyen, H.T.; Shiu, G.K.; Worsley, W.N.; Skelly, J.P. Dissolution testing of norethindrone: ethinyl estradiol, norethindrone:mestranol, and norethindrone acetate:ethinyl estradiol combination tablets. *J.Pharm.Sci.*, **1990**, *79*, 163-167

SAMPLE

Matrix: formulations

Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μ L aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μ m), discard first 5 mL of filtrate, inject a 10 μ L aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μ m), discard first 5 mL of filtrate, inject a 10 μ L aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax ODS

Mobile phase: MeOH:water 75:25

Flow rate: 1.5

Injection volume: 10**Detector:** UV 240

CHROMATOGRAM**Retention time:** 4.6 (norethindrone), 8.6 (norethindrone acetone)**Limit of detection:** 5 µg/mL

OTHER SUBSTANCES**Simultaneous:** aspirin, benzyl alcohol, benzyl benzoate, caffeine, calusterone, cortisone, dehydroepiandrosterone (UV 210), formebolone, mesterolone (UV 210), methandriol (UV 210), methandrostenolone, methenolone acetate, methyltestosterone, mibolerone, nandrolone, nandrolone acetate, nandrolone propionate, norethandrolone, norgestrel, oxymetholone, stanozolol, testolactone, testosterone, testosterone acetate, testosterone propionate, trenbolone acetate**Interfering:** boldenone, ethisterone, fluoxymesterone, oxandrolone (UV 210)

KEY WORDS

oils; tablets; suspensions

REFERENCEWalters, 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:** Dissolve 5 g cream containing 0.00925% fluocinonide, 0.00365% procinonide, and 0.0021% ciprocinonide in 2.5 mL THF, add norethindrone, dilute to 25 mL with MeOH, centrifuge, inject a 25 µL aliquot onto column A with mobile phase A and allow components to elute from column A to column B for 7 min. After 7 min remove column A from circuit, monitor effluent from column B. Back-flush column A with mobile phase B for 5 min, equilibrate column A with mobile phase A for 5 min before next injection.

HPLC VARIABLES**Column:** A 30 × 4.6 5 µm Spheri-5 ODS (Brownlee); B 70 × 2.1 Whatman Co:Pell ODS + 250 × 4.6 5 µm Ultrasphere C18**Mobile phase:** A MeCN:THF:water 43:4:53; B MeOH:THF 75:25**Flow rate:** A 1.5; B 1**Injection volume:** 25**Detector:** UV 260 for 22 min then UV 236

CHROMATOGRAM**Retention time:** 12**Internal standard:** norethindrone

OTHER SUBSTANCES**Simultaneous:** ciprocinonide, fluocinolone acetonide, fluocinonide, procinonide

KEY WORDS

creams; column-switching; norethindrone is IS

REFERENCEConley, D.L.; Benjamin, E.J. Automated high-performance liquid chromatographic column switching technique for the on-line clean-up and analysis of drugs in topical cream formulations. *J.Chromatogr.*, **1983**, *257*, 337–344

SAMPLE

Matrix: formulations

Sample preparation: 1 Tablet + 4 mL 50 mM KH_2PO_4 , rotate 15 min, add 2 mL 1 $\mu\text{g}/\text{mL}$ o-phenylphenol in mobile phase, add 4 mL MeOH, rotate 15 min, centrifuge. Remove supernatant, extract residue twice with 5 mL mobile phase (10 min rotation), combine supernatants, inject 50 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 10 μm LiChrosorb RP8

Mobile phase: MeOH:50 mM KH_2PO_4 3:2

Flow rate: 2

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Retention time: 9 (norethindrone), 19.3 (norethindrone acetate)

OTHER SUBSTANCES

Simultaneous: methyltestosterone, norgestrel

Interfering: ethinyl estradiol

KEY WORDS

tablets; stability-indicating

REFERENCE

Strusiak, S.H.; Hoogerheide, J.G.; Gardner, M.S. Determination of ethinyl estradiol in solid dosage forms by high-performance liquid chromatography. *J.Pharm.Sci.*, **1982**, *71*, 636–640

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 \times 4.6 5 μm Microsorb C8

Column: 250 \times 4.6 5 μm Microsorb C8

Mobile phase: MeCN:100 mM KH_2PO_4 adjusted to pH 7 with 1 M KOH 50:50

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8.7

Limit of detection: 100 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: 50 \times 4.6 5 μm Supelcosil LC-18

Mobile phase: MeOH:THF:water 10:20:70

Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 3 (norethindrone), 7.4 (norethindrone acetate)

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol, norethynodrel acetate, norgestrel

REFERENCE

Supelco Catalog, Supelco, Inc, Bellefonte PA, 1996, p. A130

SAMPLE

Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 $\mu\text{g}/\text{mL}$ solution in MeOH.

HPLC VARIABLES

Column: 75 \times 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: 5.941

OTHER SUBSTANCES

Simultaneous: boldenone, epimethandienone, epitestosterone, fluoxymesterone, 6 β -hydroxymethandienone, methandienone, 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: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES

Column: 250 \times 4 7 μm LichroCART RP-8 (Merck)
Mobile phase: MeCN:MeOH:water 32:37:31
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: fluoxymesterone, medrogestone, mestranol, progesterone, testosterone propionate

REFERENCE

Gau, Y.S.; Sun, S.W.; Chem, R.R.-L. Optimization of high-performance liquid chromatographic separation for progestogenic, estrogenic, and androgenic steroids using factorial design. *J.Liq.Chromatogr.*, **1995**, *18*, 2373–2382

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C18

Mobile phase: MeCN:THF:water 12.9:22.4:64.7

Flow rate: 1

Detector: UV 240

CHROMATOGRAM

Retention time: 8.5 (norethindrone), 15.5 (norethindrone acetate)

OTHER SUBSTANCES

Simultaneous: estrone, ethinyl estradiol, mestranol, norgestrel

REFERENCE

Gazdag, M.; Szepesi, G.; Szeleczi, E. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. I. Optimization for selectivity in reversed-phase chromatography. *J.Chromatogr.*, **1988**, *454*, 83–94

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb Si 60

Mobile phase: Hexane:dioxane:isopropanol 95:3:2

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 20 (norethindrone), 10 (norethindrone acetate)

OTHER SUBSTANCES

Simultaneous: estrone, ethinyl estradiol, mestranol, norgestrel

KEY WORDS

normal phase

REFERENCE

Gazdag, M.; Szepesi, G.; Fábíán-Varga, K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. II. Optimization for selectivity in normal-phase systems. *J.Chromatogr.*, **1988**, *454*, 95–107

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 240

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: hydrocortisone acetate, methyltestosterone, prednisolone, prednisolone succinate, prednisone, progesterone

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

ANNOTATED BIBLIOGRAPHY

Tang, G.P.; Chen, Q.Q. [Column switching HPLC method for determination of in vivo release of norethindrone-alpha, beta-poly (3-hydroxypropyl)-DL-asparamide conjugate]. *Yao Hsueh Hsueh Pao*, **1994**, *29*, 301–305

Lee, G.J.-L.; Oyang, M.-H.; Bautista, J.; Kushinsky, S. Determination of ethinylestradiol and norethindrone in a single specimen of plasma by automated high-performance liquid chromatography and subsequent radioimmunoassay. *J.Liq.Chromatogr.*, **1987**, *10*, 2305–2318 [LOD 20-50 pg/mL]

Papas, A.N.; Marchese, S.M.; Delaney, M.F. Rapid determination of norethindrone and ethinylestradiol in oral contraceptive tablets by reversed-phase liquid chromatography. *LC Mag.*, **1985**, *3*, 354–358 [tablets; column temp 25; simultaneous ethinylestradiol; Chem.Abs., 102, 226114]

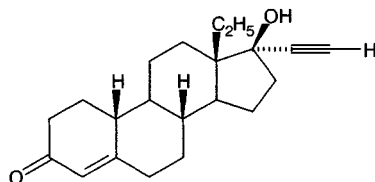
Swynnerton, N.F.; Fischer, J.B. Determination of ethynylestradiol and norethindrone in synthetic intestinal fluid and in timed-release oral formulations. *J.Liq.Chromatogr.*, **1980**, *3*, 1195–1204 [formulations; also ethinylestradiol]

Norgestrel

Molecular formula: C₂₁H₂₈O₂

Molecular weight: 312.5

CAS Registry No.: 797-63-7, 797-64-8 ((-) form), 6533-00-2



SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 3 mL MTBE, vortex for 1 min, centrifuge at 1500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μ L MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: 18 mm long (Brownlee)

Column: 300 \times 3.6 10 μ m μ Bondapak C18

Mobile phase: MeOH:water 80:20

Flow rate: 1

Detector: UV 254; RIA

CHROMATOGRAM

Retention time: 5.6

OTHER SUBSTANCES

Extracted: metabolites, norgestimate

KEY WORDS

serum

REFERENCE

Wong, F.A.; Juzwin, S.J.; Tischio, N.S.; Flor, S.C. Determination of norgestimate in serum by automated high-performance liquid chromatography and subsequent radioimmunoassay. *J.Liq.Chromatogr.*, **1995**, *18*, 1851-1861

SAMPLE

Matrix: bulk

HPLC VARIABLES

Column: 250 \times 4 10 μ m LiChrosorb RP-18

Mobile phase: MeOH:water 70:30

Flow rate: 1

Injection volume: 25

Detector: UV 240

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: impurities, norethindrone

Interfering: ethinyl estradiol

REFERENCE

Görög, S.; Herényi, B. Analysis of steroids. XXXVIII. The use of high-performance liquid chromatography with diode-array UV detection for estimating impurity profiles of steroid drugs. *J.Chromatogr.*, **1987**, *400*, 177-186

SAMPLE**Matrix:** culture medium**Sample preparation:** Extract culture medium twice with 2 volumes of ether, combine the extracts and evaporate them to dryness, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 Techopak 10 C18 (HPLC Technology)**Mobile phase:** MeOH:water 70:30**Flow rate:** 1.5**Detector:** UV 240; Radioactivity

CHROMATOGRAM**Retention time:** 5

OTHER SUBSTANCES**Extracted:** metabolites, norgestimate

KEY WORDS

tritium labeled

REFERENCE

Wild, M.J.; Rudland, P.S.; Back, D.J. Metabolism of the oral contraceptive steroids ethynylestradiol and norgestimate by normal (Huma 7) and malignant (MCF-7 and ZR-75-1) human breast cells in culture. *J.Steroid Biochem.Mol.Biol.*, **1991**, *39*, 535–543

SAMPLE**Matrix:** formulations**Sample preparation:** Centrifuge oil formulation at 30° at 2000 rpm for 30 min, filter (Whatman No. 1 paper), collect the last 4 mL of the filtrate. Dilute a 10 µL aliquot to 10 mL with MeCN:water 60:40 containing 0.3% Tween 80, remove a 2 mL aliquot and add it to 1 mL 3.33 µg/mL progesterone, vortex for 10 s, inject a 50 µL aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 Novapak C18**Mobile phase:** MeCN:water 60:40**Flow rate:** 2**Injection volume:** 50**Detector:** UV 248

CHROMATOGRAM**Internal standard:** progesterone

KEY WORDS

oils

REFERENCE

Gao, Z.-H.; Shukla, A.J.; Johnson, J.R.; Crowley, W.R. Controlled release of a contraceptive steroid from biodegradable and injectable gel formulations: In vitro evaluation. *Pharm.Res.*, **1995**, *12*, 857–863

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 \times 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: 5.9

Limit of detection: 5 $\mu\text{g/mL}$

OTHER SUBSTANCES

Simultaneous: aspirin, benzyl alcohol, benzyl benzoate, boldenone, caffeine, calusterone, cortisone, dehydroepiandrosterone (UV 210), fluoxymesterone, formebolone, mesterolone (UV 210), methandriol (UV 210), methenolone acetate, methyltestosterone, mibolerone, nandrolone acetate, nandrolone propionate, norethandrolone, norethindrone, norethindrone acetate, oxandrolone (UV 210), oxymetholone, stanozolol, testolactone, testosterone acetate, testosterone propionate, trenbolone acetate

Interfering: ethisterone, methandrostenolone, nandrolone, testosterone

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: microsomal incubations, mucosal fluid

Sample preparation: Mucosal fluid. Extract 1 mL mucosal fluid twice with 5 mL diethyl ether, evaporate extracts to dryness, resuspend residue in 100 μL MeOH, inject an aliquot. Microsomal incubations. Extract 2.5 mL microsomal preparation with 5 mL diethyl ether, proceed as before.

HPLC VARIABLES

Guard column: on-line guard column

Column: 100 \times 8 μm Bondapak radial compression module

Mobile phase: MeOH:water 70:30

Flow rate: 1.5

Injection volume: 100

Detector: UV 240

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: 3-ketonorgestimate, 17-deacetylnorgestimate, norgestimate

REFERENCE

Madden, S.; Back, D.J. Metabolism of norgestimate by human gastrointestinal mucosa and liver microsomes in vitro. *J.Steroid Biochem.Mol.Biol.*, **1991**, *38*, 497–503

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 50 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeOH:THF:water 10:20:70

Flow rate: 2

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol, norethindrone, norethindrone acetate, norethynodrel acetate

REFERENCE

Supelco Catalog, Supelco, Inc., Bellefonte PA, 1996, p. A130

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a 10 μg/mL solution.

HPLC VARIABLES

Column: 150 × 4.6 Supelco ODS

Mobile phase: MeCN:water 25:75 containing 14 mM cyclodextrin

Column temperature: 0

Flow rate: 1-3

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 16 (D(+)), 18 (D(-))

KEY WORDS

chiral

REFERENCE

Lamparczyk, H.; Zarzycki, P.K.; Nowakowska, J. Effect of temperature on separation of norgestrel enantiomers by high-performance liquid chromatography. *J.Chromatogr.A*, **1994**, 668, 413-417

SAMPLE

Matrix: solutions

Sample preparation: Direct injection.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil C-18 ODS-3

Mobile phase: MeCN:water 50:50

Flow rate: 2

Detector: UV 243

CHROMATOGRAM

Retention time: 6.0

KEY WORDS

see also *J.Pharm.Sci.* 1989, 78, 477

REFERENCE

Catz, P.; Friend, D.R. In vitro evaluations of transdermal levonorgestrel. *Drug Des.Deliv.*, **1990**, 6, 49–60

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil ODS-3 C18

Mobile phase: MeCN:water 50:50

Flow rate: 2

Detector: UV 243

CHROMATOGRAM

Retention time: 6.0

REFERENCE

Friend, D.R.; Catz, P.; Heller, J.; Okagaki, M. Transdermal delivery of levonorgestrel IV: Evaluation of membranes. *J.Pharm.Sci.*, **1989**, 78, 477–480

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Chromsep reverse phase guard column (Chrompack)

Column: 100 × 3 5 μm Chromspher glass column

Mobile phase: MeCN:water 35:65

Flow rate: 0.4

Detector: UV 247

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Simultaneous: nandrolone, trenbolone (at UV 340)

REFERENCE

Haasnoot, W.; Schilt, R.; Hamers, A.R.; Huf, F.A.; Farjam, A.; Frei, R.W.; Brinkman, U.A. Determination of β-19-nortestosterone and its metabolite α-19-nortestosterone in biological samples at the sub parts per billion level by high-performance liquid chromatography with on-line immunoaffinity sample pretreatment. *J.Chromatogr.*, **1989**, 489, 157–171

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Nucleosil C18

Mobile phase: MeCN:THF:water 12.9:22.4:64.7

Flow rate: 1

Detector: UV 240

CHROMATOGRAM**Retention time:** 13

OTHER SUBSTANCES**Simultaneous:** estrone, ethinyl estradiol, mestranol, norethindrone, norethindrone acetate

REFERENCE

Gazdag, M.; Szepesi, G.; Szelezcki, E. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. I. Optimization for selectivity in reversed-phase chromatography. *J.Chromatogr.*, **1988**, *454*, 83-94

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 5 μm LiChrosorb Si 60**Mobile phase:** Hexane:dioxane:isopropanol 95:3:2**Flow rate:** 1**Detector:** UV 254

CHROMATOGRAM**Retention time:** 13

OTHER SUBSTANCES**Simultaneous:** estrone, ethinyl estradiol, mestranol, norethindrone, norethindrone acetate

KEY WORDSnormal phase

REFERENCE

Gazdag, M.; Szepesi, G.; Fábíán-Varga, K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. II. Optimization for selectivity in normal-phase systems. *J.Chromatogr.*, **1988**, *454*, 95-107

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 μBondapak C18**Mobile phase:** Dioxane:water 50:50 (Caution! Dioxane is a carcinogen!)**Flow rate:** 1.4**Detector:** UV 254

OTHER SUBSTANCES**Simultaneous:** norgestimate

REFERENCE

Killinger, J.; Hahn, D.W.; Phillips, A.; Heteyi, N.S.; McGuire, J.L. The affinity of norgestimate for uterine progesterone receptors and its direct action on the uterus. *Contraception*, **1985**, *32*, 311-319

SAMPLE**Matrix:** tissue**Sample preparation:** Incubate endometrial tissue with buffer, remove tissue, extract medium twice with 2 volumes of diethyl ether, evaporate to dryness, reconstitute in a small volume of MeOH, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Technopak 10 C18

Mobile phase: MeOH:water 70:30

Flow rate: 1.5

Detector: UV 240

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: metabolites, norgestimate

KEY WORDS

endometrial tissue

REFERENCE

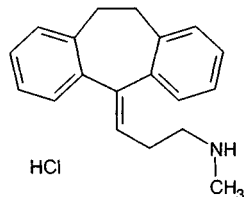
Wild, M.J.; Rudland, P.S.; Back, D.J. Metabolism of the oral contraceptive steroids ethynylestradiol, norgestimate and 3-ketodesogestrel by a human endometrial cancer cell line (HEC-1A) and endometrial tissue *in vitro*. *J.Steroid Biochem.Mol.Biol.*, **1993**, *45*, 407–420

Nortriptyline

Molecular formula: C₁₉H₂₁N

Molecular weight: 263.4

CAS Registry No.: 72-69-5, 894-71-3 (HCl)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 500 μ L 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μ L 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 μ L 22 mM pH 2.5 KH₂PO₄/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 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 \times 4.6 Supelco C18

Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH₂PO₄ 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: 9.73

Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, citalopram

Simultaneous: chlorprothixene, clomipramine, clozapine, flupenthixol, haloperidol, levomepromazine, perphenazine, zuclopenthixol

Noninterfering: benzodiazepines

Interfering: desmethyllevomepromazine

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: blood

Sample preparation: 1 mL Plasma + doxepin + 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 \times 8 10 μ m 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: 31
Internal standard: doxepin (11.6)

OTHER SUBSTANCES

Extracted: amitriptyline, fluoxetine, norfluoxetine

KEY WORDS

plasma

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

Sample preparation: 200 μ L Plasma or serum + 100 μ L 2 M pH 10.6 Tris buffer + 200 μ L MTBE, vortex for 30 s, centrifuge at 9950 g for 3 min, inject a 50 μ L aliquot of the organic layer.

HPLC VARIABLES

Guard column: 10 \times 4.6 SCX-10C5 (Hichrom)

Column: 150 \times 4.6 Spherisorb S5 SCX (sulfopropyl-bonded silica) cation exchange

Mobile phase: 35 mM ammonium perchlorate in MeOH adjusted to an apparent pH of 6.7 with 100 mM NaOH in MeOH

Flow rate: 1.5

Injection volume: 50

Detector: UV 215

CHROMATOGRAM

Retention time: 9

Internal standard: nortriptyline hydrochloride

OTHER SUBSTANCES

Extracted: clozapine

Simultaneous: amitriptyline, chlorpromazine, clomipramine, dothiepin, doxepin, fluoxetine, fluphenazine, fluvoxamine, haloperidol, imipramine, maprotiline, mianserin, nortriptyline, nortriptyline, nordoxepin, norfluoxetine, nortriptyline, paroxetine, remoxipride, sulpride, thioridazine, trazodone

Noninterfering: carbamazepine, clonazepam, diazepam, flunitrazepam, lorazepam, nordiazepam, theophylline

Interfering: sertraline

KEY WORDS

plasma; serum; nortriptyline is IS

REFERENCE

McCarthy, P.T.; Hughes, S.; Paton, C. Measurement of clozapine and nortriptyline in plasma/serum by high-performance liquid chromatography with ultraviolet detection. *Biomed.Chromatogr.*, **1995**, *9*, 36–41

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)) KH₂PO₄ 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 239

CHROMATOGRAM**Retention time:** 9.28**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES**Extracted:** acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, fenfluramine, fenpropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, medazepam, medifoxamine, mefenamic acid, mefenidramine, melfhalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfipyrzazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, triprolidine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: amitriptyline, daunorubicin, diclofenac, etodolac, fluoxetine, indomethacin, maprotiline, mefloquine, tiocloमारol, trimipramine, tropatenine

KEY WORDS

whole blood; plasma

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 + 500 μ L 250 μ g/mL protriptyline hydrochloride + 1 mL 500 mM NaOH + 4 mL toluene:n-hexane:isoamyl alcohol 77:22:3, mix for 10 min, centrifuge at 3000 rpm for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μ L MeOH, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.7 5 μ m Supelcosil LC-PCN cyanopropyl

Mobile phase: MeCN:MeOH:10 mM pH 7.2 potassium phosphate buffer 60:15:25 (Prepare buffer by mixing 194 mL 1.36 g/L KH_2PO_4 with 274 mL 1.74 g/L K_2HPO_4)

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 6.95

Internal standard: protriptyline (8.1)

OTHER SUBSTANCES

Extracted: amitriptyline, cyclobenzaprine

Interfering: norcyclobenzaprine

KEY WORDS

serum

REFERENCE

Wong, E.C.C.; Koenig, J.; Turk, J. Potential interference of cyclobenzaprine and norcyclobenzaprine with HPLC measurement of amitriptyline and nortriptyline: resolution by GC-MS analysis. *J. Anal. Toxicol.*, **1995**, *19*, 218-224

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 the SPE cartridge, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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: 4.8

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, 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, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocanide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: imipramine, maprotiline, verapamil

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: 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 μ L Serum + 50 μ L 5 μ g/mL trimipramine in 5% potassium bicarbonate + 700 μ L 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 μ L MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μ L aliquot of the eluate.

HPLC VARIABLES

Guard column: 15 mm 7 μ m Brownlee RP-8

Column: 150 \times 4.6 5 μ m 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:** 7.4**Internal standard:** trimipramine (9.6)**Limit of quantitation:** 5 ng/mL

OTHER SUBSTANCES**Extracted:** amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, maprotiline, protriptyline**Interfering:** imipramine

KEY WORDS

serum; SPE

REFERENCEGupta, 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:** 500 μ L Serum + 250 μ L 100 mM lauryl sulfate, centrifuge at 2500 g for 8 min, inject a 250 μ L aliquot of the supernatant onto column A with mobile phase A, elute with mobile phase A for 6 min, backflush contents of column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B for 6 min and conduct analysis. When not in use flush column A with mobile phase A. Every eight injections backflush column A with MeCN:water 70:30.

HPLC VARIABLES**Column:** A Guard-Pak 10 μ m Resolve CN (Waters); B 150 \times 3 7 μ m Separon SGX CN (Tessek)**Mobile phase:** A MeCN:water 3:97; B MeCN:buffer 26:74 (Buffer was 50 mM phosphoric acid, 50 mM ammonium phosphate, and 28 mM diethylamine, pH 2.55.)**Flow rate:** 1**Injection volume:** 250**Detector:** UV 210

CHROMATOGRAM**Retention time:** 10**Limit of detection:** 15-20 ng/mL

OTHER SUBSTANCES**Extracted:** amitriptyline

KEY WORDS

serum; column-switching

REFERENCEDolezalová, M. On-line solid-phase extraction and high-performance liquid chromatographic determination of nortriptyline and amitriptyline in serum. *J.Chromatogr.*, **1992**, *579*, 291–297

SAMPLE**Matrix:** blood**Sample preparation:** Add 10 μ L 20 μ g/mL oxaprotiline in MeOH to 990 μ L plasma or serum. Inject 100 μ L 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: 14.5

Internal standard: oxaprotiline (9.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, clozapine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, metoclopramide, norfluoxetine

Noninterfering: carbamazepine, chlordiazepoxide, clobazam, diazepam, flurazepam, fluspirilene, haloperidol, lorazepam, nitrazepam, nordiazepam, oxazepam, perazine, pimozide, spiroperidol, trifluoperidol

KEY WORDS

plasma; serum; column-switching

REFERENCE

Härtter, S.; Wetzels, 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: 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 K₂HPO₄ adjusted to pH 6.8 with 85% phosphoric acid.)

Flow rate: 1.5

Injection volume: 100

Detector: UV 214

CHROMATOGRAM

Retention time: 14.54

Internal standard: trimipramine (6.5)

Limit of detection: 5-10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, clomipramine, desipramine, doxepin, fluvoxamine, imipramine, maprotiline

Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluoperidol

KEY WORDS

plasma; serum; column-switching; LOD can be lowered to 1 ng/mL with 3 injections onto column A before 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: 1 mL Plasma + 100 μ L 1 M pH 7.6 K_2HPO_4 , vortex for 5 s, add 6 mL ethyl acetate, vortex for 1 min, centrifuge at 1900 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μ L 40 mM sodium bicarbonate, add 20 μ L 10 mg/mL dansyl chloride in acetone, add 750 μ L acetone, vortex for 30 s, let stand at room temperature at 22° for 15 min. Evaporate under a stream of nitrogen at 45°, reconstitute in 1 mL mobile phase, vortex for 1 min, centrifuge at 1900 g for 10 min, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m NewGuard RP-8 (Brownlee)

Column: 250 \times 4.6 5 μ m Supelcosil LC-18-DB

Mobile phase: MeCN:10 mM pH 7.2 potassium phosphate 85:15

Flow rate: 1.5

Injection volume: 100

Detector: F (wavelengths not given)

CHROMATOGRAM

Retention time: 9.7

Internal standard: nortriptyline

OTHER SUBSTANCES

Extracted: fluvoxamine

Simultaneous: desipramine

KEY WORDS

plasma; derivatization; nortriptyline is IS

REFERENCE

Pullen, R.H.; Fatmi, A.A. Determination of fluvoxamine in human plasma by high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1992**, *574*, 101–107

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μ L 5 μ g/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 μ L 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 μ L 1 M pH 10.3 carbonate buffer and 25 μ L 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 \times 4.6 5 μm Supelcosil LC-18

Mobile phase: MeCN:25 mM KH_2PO_4 75:25 containing 500 $\mu\text{L/L}$ orthophosphoric acid and 600 $\mu\text{L/L}$ n-butylamine

Flow rate: 2

Injection volume: 25-40

Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM

Retention time: 14.18

Internal standard: maprotiline (12.8)

OTHER SUBSTANCES

Simultaneous: amoxapine, clovoxamine, desipramine, fenfluramine, fluoxetine, fluvoxamine, norfluoxetine, propranolol, protriptyline, sertraline

Noninterfering: amitriptyline, atenolol, bupropion, carbamazepine, chlordiazepoxide, citalopram, clomipramine, clozapine, cyclobenzaprine, doxepin, imipramine, loxapine, metoprolol, mianserin, moclobemide, nomifensine, pindolol, thioridazine, tranlycypromine, trazodone, trimipramine

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: 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 \times 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' 2.460

Internal standard: clobazam (k' 1.344)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, desipramine, imipramine

Simultaneous: alprazolam, clonazepam, diazepam, flunitrazepam, haloperidol, lorazepam, maprotiline, nitrazepam, triazolam

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: 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 \times 4 TSKprecolumn PW (Tosoh); B 150 \times 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:** 15**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** amitriptyline, amoxapine, clomipramine, doxepin, desipramine, maprotiline, trimipramine**Interfering:** imipramine

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 Plasma + 50 μL MeOH:water 50:50 + 50 μL 10 $\mu\text{g}/\text{mL}$ desmethyldoxepin in MeOH:water 50:50, mix, inject a 250 μL aliquot of this mixture onto column A and elute to waste with mobile phase A. After 1.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES**Column:** A 37-50 μm 10 \times 1.5 Corasil RP C18; B 100 \times 4 Techsphere 3CN (HPLC Technology)**Mobile phase:** A water; B MeCN:50 mM acetate buffer 60:40, pH 7**Flow rate:** A 0.8; B 0.9**Injection volume:** 250**Detector:** UV 215

CHROMATOGRAM**Retention time:** 10.0**Internal standard:** desmethyldoxepin (8.5)**Limit of detection:** 5-10 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites, amitriptyline**Simultaneous:** chloripramine, clomipramine, desipramine, doxepin, imipramine, protriptyline**Noninterfering:** tranlycypromine**Interfering:** cianopramine, trimipramine

KEY WORDScolumn-switching; plasma

REFERENCE

Dadgar, D.; Power, A. Applications of column-switching technique in biopharmaceutical analysis. I. High-performance liquid chromatographic determination of amitriptyline and its metabolites in human plasma. *J.Chromatogr.*, **1987**, *416*, 99-109

SAMPLE**Matrix:** blood**Sample preparation:** Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μ L Serum + 50 μ L 1 μ g/mL N-propionylprocainamide in 2.5 mM HCl, add to the 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 μ L 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 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES**Guard column:** Pelliguard LC-CN (Supelco)**Column:** 150 \times 4.6 5 μ m 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:** 14.0**Internal standard:** N-propionylprocainamide (6)**Limit of quantitation:** 25 ng/mL

OTHER SUBSTANCES**Extracted:** amitriptyline, desipramine, doxepin, imipramine, 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, meprobital, 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: 500 μ L Serum + 250 μ L di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μ L aliquot of top organic layer.

HPLC VARIABLES

Guard column: 30 \times 4.6 5 μ m Brownlee cyano spheri-5

Column: 250 \times 4.6 5 μ m 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 240

CHROMATOGRAM

Retention time: 6.5

Internal standard: minaprine (5.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, diltiazem

Also analyzed: amiodarone, clomipramine, desipramine, haloperidol, imipramine, propafenone, 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: 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 μ L 200 mM HCl, shake for 2 min, centrifuge. Inject a 20 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 100 \times 3 octyl CP-tm-Spher C8 glass column (Chrompack)

Mobile phase: MeCN:500 mM NaH₂PO₄ 35:65 adjusted to pH 2.2 with phosphoric acid

Flow rate: 0.8

Injection volume: 20

Detector: UV 210

CHROMATOGRAM

Retention time: 4.5

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, doxepin

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: 2 mL Serum or plasma + 100 μ L 1 μ g/mL IS in water + 0.5 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 μ L mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 μ L aliquot of the lower layer.

HPLC VARIABLES

Column: 250 \times 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: 8.4

Internal standard: U-31485 (6.9)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: desipramine, protriptyline

Noninterfering: N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine

Interfering: alprazolam, imipramine, triazolam

KEY WORDS

serum; plasma

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 μ L Plasma + 37 μ L 2 μ g/mL IS in MeOH + 500 μ L 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 μ L MeOH, inject a 50 μ L 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: 9

Internal standard: N-desmethylclomipramine hydrochloride (10)

Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, desipramine, 2-hydroxydesipramine, 2-hydroxyimipramine, imipramine

Also analyzed: clomipramine, desmethylclomipramine, desmethyldoxepin, doxepin, 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 tetra-cyclic antidepressants and their major active metabolites. *Clin.Chem.*, **1984**, *30*, 471-474

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

Mobile phase: 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine (EtOH:MeCN:t-butylamine 98:2:0.05)

Flow rate: 2

Injection volume: 75-100

Detector: UV 254

CHROMATOGRAM

Retention time: 6.9

Internal standard: promazine (5.2)

Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, desmethyldoxepin, doxepin, imipramine, protriptyline

Simultaneous: N-acetylprocainamide, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorimipramine, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desmethylchlordiazepoxide, desmethyldisopyramide, dextropropoxyphene, diazepam, di-

sopyramide, fluphenazine, hydroxyamoxapine, 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, iprindole, loxepin, maprotiline, meperidine, methadone, mianserin, morphine, norzimeldine, oxapam, oxaprotiline, perphenazine, phenteramine, procainamide, prolixin, promethazine, propoxyphene, pyrilamine, quinidine, thioridazine, trifluoperazine, trifluopromazine, trimeprazine, trimipramine, zimeldine

Noninterfering: thiopropazine

Interfering: prochlorperazine

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 μ L 1 μ g/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μ L 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 μ L 250 mM HCl, vortex for 30 s, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 \times 4.6 40 μ m C8 (Supelco)

Column: 250 \times 4.6 5 μ m 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' 3.45

Internal standard: loxapine (k' 7.18)

Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, imipramine, oxazepam

Noninterfering: molindone, perphenazine, trifluoperazine

Interfering: haloperidol, thiothixene

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: 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 μ L mobile phase, inject a 50 μ L aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μ m 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: 10.7

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, trifluorpromazine, 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: 1 mL Serum + 200 μ L 10 μ g/mL protriptyline in water + 200 μ L 80 g/L NaHCO₃ + 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 μ L mobile phase, vortex for 15 s, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak 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: 7.10

Internal standard: protriptyline (12.20)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, chlorpromazine, desipramine, desmethyldoxepin, doxepin, imipramine, maprotiline, procainamide, propoxyphene, propranolol, thioridazine, trimipramine

Noninterfering: acetaminophen, caffeine, chlordiazepoxide, diazepam, methaqualone, salicylic acid, theophylline, trifluoperazine

Interfering: disopyramide

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: 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 μ L 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: μ Bondapak/Porasil

Column: μ Bondapak 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: 4.5

Internal standard: clomipramine (7.5)

Limit of detection: 0.6 ng

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, imipramine

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 antidepressants in plasma. *J.Liq.Chromatogr.*, **1981**, *4*, 849-862

SAMPLE

Matrix: blood, dialysate

Sample preparation: Adjust pH of serum samples to 7.4 and dialyze 3 mL serum against 5 mL dialysis buffer at 37° in PTFE chambers for 4 h. Inject 1 mL mobile phase, 1 mL water, 2 mL dialysate, 1 mL water, and 1 mL MeCN:water 50:50 onto column A. Elute column A onto column B with mobile phase for 30 s then remove it from the circuit. Elute column B with mobile phase and monitor the effluent. (Dialysis buffer was 3.998 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ + 0.775 g NaH_2PO_4 + 2.250 g NaCl + 0.055 g $\text{Hg}(\text{NO}_3)_2$ in 1 L water, pH was 7.4.)

HPLC VARIABLES

Column: A 10×6 packed with $40 \mu\text{m}$ material from a Bond Elut cartridge (cat. no. 620303);
B $100 \times 4.3 \mu\text{m}$ Spherisorb ODS Superpac
Mobile phase: MeCN:85% phosphoric acid:triethylamine:water 49.55:0.225:0.225:50
Flow rate: 0.65
Injection volume: 1000-2000
Detector: UV 238

CHROMATOGRAM

Retention time: 3.85
Limit of detection: 0.5 nM (5 mL sample)

OTHER SUBSTANCES

Extracted: amitriptyline

Simultaneous: alprazolam, chlorpromazine, chlorprothixene, clomipramine, desclomipramine, desmethylimipramine, diazepam, flunitrazepam, fluphenazine, haloperidol, imipramine, levomepromazine, perphenazine, protriptyline, thioridazine, thioridazine sulfone, trimipramine, zimeldine, zuclopenthixol

Noninterfering: carbamazepine, clonazepam, lorazepam, nitrazepam, oxazepam, phenytoin

Interfering: maprotiline, promethazine, thioridazine sulfoxide

KEY WORDS

serum; column-switching

REFERENCE

Svensson, C.; Nyberg, G.; Mårtensson, E. High-performance liquid chromatographic quantitation of amitriptyline and nortriptyline in dialysate from plasma or serum using on-line solid-phase extraction. *J. Chromatogr.*, **1988**, *432*, 363-369

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: $4 \times 4.30 \mu\text{m}$ LiChrocart Aluspher RP-select B (Merck)
Column: $125 \times 4.5 \mu\text{m}$ 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; UV 254

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, pindolol, zolpidem

Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodaine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylamine, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyl dopa, 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, tripeleppamine, 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: Whole blood, serum. 1 mL Whole blood or serum + 1 µg cyanopramine + 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. Tissue. 0.5 mL Liver homogenate + 10 µg cyanopramine + 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 NaH₂PO₄:diethylamine 40:57.5:2.5

Flow rate: 2

Injection volume: 30

Detector: UV 220

CHROMATOGRAM

Retention time: 7.27

Internal standard: cyanopramine (8.93)

Limit of detection: 50 ng/mL

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, norfluoxetine, norpropoxyphene, northiaden, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulfuridazine, thioridazine, thiothixene, tranlycypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphethidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: diphenhydramine

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: Serum. 1 mL Serum + 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 μ L mobile phase buffer:MeOH 9:1, inject 100 μ L aliquot. Tissue. 2 g Brain tissue + 10 mL 2.5% perchloric acid + 8 mL MeOH, 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 μ L mobile phase buffer:MeOH 9:1, inject 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 14.2

Internal standard: nortriptyline

Limit of detection: 10 ng/g (tissue)

OTHER SUBSTANCES

Extracted: desipramine, imipramine

KEY WORDS

serum; rat; nortriptyline is IS

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: 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 μ g 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 μ L 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m 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 NaH₂PO₄ + 30 mL diethylamine.)

Flow rate: 2

Injection volume: 30

Detector: UV 214

CHROMATOGRAM

Retention time: 5

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, desipramine, dothiepin, doxepin, haloperidol, imipramine, mianserin

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: 250 μL Microsomal incubation + 50 μL 1 M HCl, cool on ice, add desipramine, centrifuge at 16000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:50 mM pH 6 potassium phosphate buffer 35:65

Flow rate: 1.4

Detector: UV 220

CHROMATOGRAM

Retention time: 10.5

Internal standard: desipramine (9)

Limit of detection: LOD 50 pmole

OTHER SUBSTANCES

Extracted: amitriptyline

Noninterfering: ketoconazole, α-naphthoflavone, quinidine

KEY WORDS

human; liver

REFERENCE

Schmider, J.; Greenblatt, D.J.; von Moltke, L.L.; Harmatz, J.S.; Shader, R.I. N-Demethylation of amitriptyline *in vitro*: Role of cytochrome P-450 3A (CYP3A) isoforms and effect of metabolic inhibitors. *J.Pharm.Exp.Ther.*, **1995**, *275*, 592–597

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: 13.70 (A), 6.75 (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, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, 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, sulfipyrazone, 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

some 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

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: 13.8

OTHER SUBSTANCES

Simultaneous: amitriptyline, desipramine, desmethyldoxepin, doxepin, imipramine

Also analyzed: amphetamine, chlordiazepoxide, chlorpromazine, desalkylflurazepam, diazepam, diethylpropion, ephedrine, fenfluramine, flurazepam, mesoridazine, methamphetamine, norchloriazepoxide, nordiazepam, oxazepam, phentermine, phenylpropanolamine, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog Ci-94, 1994-5, Rainin Instrument Co., Woburn MA, 1994, p. 7.24

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 4.03

OTHER SUBSTANCES

Simultaneous: amitriptyline, benactyzine, buclizine, desipramine, hydroxyzine, imipramine, perphenazine, protriptyline, thioridazine

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 \times 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, bromazepam, 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, dapson, 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, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naprofen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, 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, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, 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 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 KH₂PO₄ 23.4:76.6 containing 0.05% nonylamine

Flow rate: 1.2

Detector: UV 214

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: amitriptyline, desmethyldoxepin, desipramine, doxepin, imipramine, loxapine, maprotiline, trazodone

REFERENCE

Alltech Chromatography Catalog 300, Alltech Associates, Inc., Deerfield IL, 1993, p. 440

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 250 × 4.6 Econosil C8

Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH₂PO₄ and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)

Injection volume: 20

Detector: UV 210

CHROMATOGRAM

Retention time: 9.9

Limit of quantitation: < 1 μg/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, carbamazepine, imipramine

Also analyzed: desipramine, cyclobenzaprine, doxepin, maprotiline, protriptyline

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

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' 2.60

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**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, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mepatazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, penethiate, pericyazine, perphenazine, phenadoxone, phenampramide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phenotolamine, 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, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleannamine, 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: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 4 Lichrospher 5 μm 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: 15

OTHER SUBSTANCES

Extracted: benzoylcegonine, cocaine, diphenhydramine, ephedrine, lidocaine, morphine, nordiazepam, norpropoxyphene, phenylpropanolamine

Also analyzed: amitriptyline, amphetamine, codeine (different gradient), meperidine

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: 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 start 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, 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 \times 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 \times 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 \times 3.2 5 μm C8 (Phenomenex) + 150 \times 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: 40 (B, C only)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210; UV 235

CHROMATOGRAM

Retention time: k' 3.3

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: amphetamine, benzoylecgonine, caffeine, cotinine, diazepam, ephedrine, lidocaine, nordiazepam, oxazepam, pentazocine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, secobarbital, amitriptyline, codeine, flurazepam, hydrocodone, hydromorphone, imipramine, methadone, morphine

Interfering: desipramine, diphenhydramine, methamphetamine

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: vitreous humor

Sample preparation: 600 μL Vitreous humor + 3 mL 0.1 M NaCl + 50 μL 4 $\mu\text{g}/\text{mL}$ 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 with 3 mL reagent by gravity. Elute with 3 mL MeOH and push air through to remove as much as possible. Evaporate eluate 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 \times 4.6 30 μm Permaphase ETH

Column: 250 \times 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: 20.5

Internal standard: desmethylclomipramine

Limit of detection: 16.7 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, amitriptyline, doxepin, imipramine

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, diazepam, digoxin, disopyramide, ethosuximide, flurazepam, gentamicin, haloperidol, kanamycin, lidocaine, meprobamate, methapyrilone, 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

ANNOTATED BIBLIOGRAPHY

Atta-Politou, J.; Tsarpalis, K.; Koutselinis, A. A modified simple and rapid reversed phase high performance liquid chromatographic method for quantification of amitriptyline and nortriptyline in plasma. *J.Liq.Chromatogr.*, **1994**, *17*, 3969–3982 [extracted amitriptyline, clomipramine, doxepin, protriptyline; LOD 5 ng/mL; LOQ 10 ng/mL; interfering phenytoin; simultaneous chlordiazepoxide, chlorpheniramine, chlorpromazine, imipramine, maprotiline, phenobarbital, propoxyphene, propranolol; non-interfering alprazolam, artane, bromazepam, diazepam, haloperidol, lorazepam, nitrazepam, oxazepam, pseudoephedrine, theophylline, triazolam]

Oshima, N.; Kotaki, H.; Sawada, Y.; Iga, T. Tissue distribution of amitriptyline after repeated administration in rats. *Drug Metab.Dispos.*, **1994**, *22*, 21–25 [rat; dialysate; plasma; tissue; liver; kidney; lung; brain; muscle; heart; clomipramine (IS); column temp 35; pharmacokinetics]

Piperaki, S.; Parissi-Poulou, M.; Koupparis, M. A separation study of tricyclic antidepressant drugs by HPLC with β -cyclodextrin bonded stationary phase. *J.Liq.Chromatogr.*, **1993**, *16*, 3487–3508 [also amitriptyline, chloripramine, doxepin, imipramine, maprotiline, protriptyline]

Smith, C.S.; Abramson, R.K.; Morgan, S.L. An investigation of the metabolism of amitriptyline using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1986**, *9*, 1727–1745 [extracted amitriptyline; rat; tissue; liver]

Wong, S.H.Y.; McHugh, S.L.; Dolan, J.; Cohen, K.A. Tricyclic antidepressant analysis by reversed-phase liquid chromatography using phenyl columns. *J.Liq.Chromatogr.*, **1986**, *9*, 2511–2538 [also acetaminophen, amitriptyline, amobarbital, amoxapine, barbital, chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, codeine, desipramine, desmethyldoxepin, diazepam, doxepin, fluphenazine, flurazepam, glutethimide, hydroxyamoxapine, imipramine, lorazepam, maprotiline, meperidine, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, propoxyphene, protriptyline, secobarbital, thioridazine, trazodone]

Suckow, R.F.; Cooper, T.B. Simultaneous determination of amitriptyline, nortriptyline and their respective isomeric 10-hydroxy metabolites in plasma by liquid chromatography. *J.Chromatogr.*, **1982**, *230*, 391–400

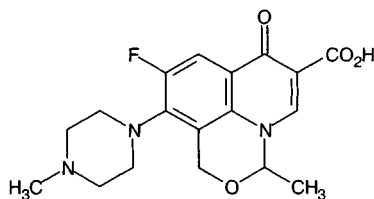
Jensen, K.M. Determination of amitriptyline-N-oxide, amitriptyline and nortriptyline in serum and plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1980**, *183*, 321–329

Ofloxacin

Molecular formula: C₁₈H₂₀FN₃O₄

Molecular weight: 361.4

CAS Registry No.: 82419-36-1



SAMPLE

Matrix: blood

Sample preparation: 250 μ L Serum + 50 μ L pipemidic acid solution + 200 μ L chloroform, extract. Extract the organic phase with 200 μ L 100 mM NaOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: C18

Mobile phase: MeCN:buffer 13:87 (Buffer was 10 mM NaH₂PO₄ and 5 mM tetrabutylammonium hydrogen sulfate, pH 2.7.)

Flow rate: 1.4

Injection volume: 20

Detector: F ex 282

CHROMATOGRAM

Internal standard: pipemidic acid

Limit of quantitation: 50 ng/mL

KEY WORDS

serum; pharmacokinetics

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

Sample preparation: 1 mL Plasma + 50 μ L 180 μ g/mL enoxacin in MeOH, shake briefly, add 3 mL MeCN, shake at 1000 rpm for 30 s, centrifuge at 2600 g for 10 min. Remove 3 mL of the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μ L 60 mM KH₂PO₄ adjusted to pH 2.6 with orthophosphoric acid, inject a 30 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.7 μ m Separon SGX C18 (Tessek, Prague)

Mobile phase: THF:buffer 5.5:94.5 (Buffer was 60 mM KH₂PO₄ adjusted to pH 2.6 with orthophosphoric acid:triethylamine 97:3 containing 50 μ g/mL sodium azide. Caution! Sodium azide is carcinogenic and toxic and must not be discharged to the plumbing system!)

Flow rate: 0.7

Injection volume: 30

Detector: F ex 282 em 450

CHROMATOGRAM

Retention time: 6.7

Internal standard: enoxacin (5.0)

Limit of detection: 8 ng/mL

Limit of quantitation: 20 ng/mL

KEY WORDS

plasma

REFERENCE

Macek, J.; Ptáček, P. Determination of ofloxacin in human plasma using high-performance liquid chromatography and fluorescence detection. *J.Chromatogr.B*, **1995**, 673, 316–319

SAMPLE**Matrix:** blood

Sample preparation: 500 μ L Serum + 100 μ L water + 1 mL MeCN, shake mechanically for 30 s, centrifuge at 10000 g for 5 min. Remove 1.5 mL of the supernatant and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 300 μ L mobile phase, inject a 25 μ L aliquot.

HPLC VARIABLES**Guard column:** 30 \times 4 30-40 μ m Perisorb RP-18 (Merck)**Column:** 125 \times 4 5 μ m Nucleosil 100 SA

Mobile phase: MeCN:buffer 75:25, adjusted to pH 3.82 with concentrated phosphoric acid, final sodium concentration 23 mM (Buffer was 6.74 mL concentrated phosphoric acid and 40 mL 2 M NaOH made up to 990 mL with water, adjust pH to 2.92 with concentrated phosphoric acid, make up to 1 L with water.)

Flow rate: 1.5**Injection volume:** 25**Detector:** F ex 295 em 525

CHROMATOGRAM**Retention time:** 8.0**Internal standard:** ofloxacin

OTHER SUBSTANCES**Extracted:** sparfloxacin

KEY WORDS

ofloxacin is IS; serum; protect from light

REFERENCE

Borner, K.; Borner, E.; Lode, H. Determination of sparfloxacin in serum and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1992**, 579, 285–289

SAMPLE**Matrix:** blood

Sample preparation: 500 μ L Serum + 500 μ L 7% perchloric acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** 100 \times 8 μ Bondapak C18 Radial-PAK

Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1

Injection volume: 20**Detector:** F ex 294 em 475

CHROMATOGRAM**Retention time:** 5.6

KEY WORDS

serum

REFERENCE

Griggs, D.J.; Wise, R. A simple isocratic high-pressure liquid chromatographic assay of quinolones in serum. *J.Antimicrob.Chemother.*, **1989**, *24*, 437-445

SAMPLE

Matrix: blood

Sample preparation: 50 μ L Plasma + 1 mL 100 mM pH 7.0 K_2HPO_4 adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μ L 300 μ g/mL nalidixic acid in water + 3 mL dichloromethane:isoamyl alcohol 9:1, shake vigorously for 10 min, centrifuge at 2270 g for 10 min. Remove 2 mL of the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute residue in 100 μ L MeOH:50 mM NaOH 2:1, vortex, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Chemcosorb 5-ODS-H

Mobile phase: MeOH:5 mM sodium lauryl sulfate 2:1, adjusted to pH 2.5 with 85% phosphoric acid (Better separation obtained at pH 2.35, *J.Chromatogr.* 1990, 530, 186.)

Column temperature: 40

Flow rate: 0.6

Injection volume: 10

Detector: UV 300

CHROMATOGRAM

Retention time: 5.9

Internal standard: nalidixic acid (5.0)

Limit of detection: 150 ng/mL

OTHER SUBSTANCES

Extracted: felbinac, fenbufen, norfloxacin

Interfering: enoxacin

KEY WORDS

plasma; rat

REFERENCE

Katagiri, Y.; Naora, K.; Ichikawa, N.; Hayashibara, M.; Iwamoto, K. Simultaneous determination of ofloxacin, fenbufen and felbinac in rat plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *431*, 135-142

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 2 mL 100 mM pH 7.0 phosphate buffer + 100 μ L 1 mg/mL flufenamic acid in phosphate buffer + 6 mL chloroform, agitate on a rotary mixer for 15 min, centrifuge at 4000 g at 4° for 5 min. Remove the organic phase and evaporate it to dryness under nitrogen at 37°. Suspend the residue in 100 μ L 100 mM pH 7.0 phosphate buffer, vortex for 45 s, centrifuge at 9000 g for 2 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m Hypersil ODS

Mobile phase: MeCN:40 mM phosphoric acid 45:55, final pH 2.56-2.59

Column temperature: 17

Flow rate: 2

Injection volume: 20

Detector: F ex 370 em 400-700

CHROMATOGRAM**Retention time:** 3.83**Internal standard:** flufenamic acid (2.67)**Limit of quantitation:** 2.5 ng/mL

KEY WORDSplasma

REFERENCE

Notarianni, L.J.; Jones, R.W. Method for the determination of ofloxacin, a quinolone carboxylic acid antimicrobial, by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *431*, 461-464

SAMPLE**Matrix:** blood, CSF

Sample preparation: Serum. 1 mL Serum + 500 μ L 500 mM pH 7 phosphate buffer + 8 mL dichloromethane, rotate for 10 min at 20 rpm, centrifuge at 4000 rpm for 15 min. Remove the organic layer and evaporate it to dryness at 40° in a vortex evaporator. Dissolve in 1 mL mobile phase, inject a 100 μ L aliquot. CSF. Inject a 20 μ L aliquot directly.

HPLC VARIABLES**Column:** 250 \times 4.5 μ m Nucleosil C18**Mobile phase:** MeCN:40 mM pH 2.2 phosphate buffer containing tetrabutylammonium chloride as the ion-pairing reagent 15:85**Flow rate:** 1**Injection volume:** 20 (CSF), 100 (serum)**Detector:** F ex 295 em 475

CHROMATOGRAM**Limit of detection:** 90 ng/mL (serum); 70 ng/mL (CSF)

KEY WORDSserum

REFERENCE

Bitar, N.; Claes, R.; Van der Auwera, P. Concentrations of ofloxacin in serum and cerebrospinal fluid of patients without meningitis receiving the drug intravenously and orally. *Antimicrob.Agents Chemother.*, **1989**, *33*, 1686-1690

SAMPLE**Matrix:** blood, intestinal efflux

Sample preparation: Intestinal efflux. Freeze intestinal efflux at -80°, lyophilize, reconstitute with 1 mL MeOH:100 mM phosphoric acid 50:50, centrifuge at 3000 rpm for 10 min, inject a 20 μ L aliquot. Serum. Deproteinize serum with MeOH.

HPLC VARIABLES**Column:** 150 \times 3.9 Novapack C18**Mobile phase:** MeOH:buffer 25:75 (Buffer was 10 mM pH 3.0 potassium phosphate buffer containing 25 mM sodium heptanesulfonate (PIC B7) and 20 mM triethylamine.)**Flow rate:** 1.5**Injection volume:** 20**Detector:** F ex 330 em 440

CHROMATOGRAM**Internal standard:** ofloxacin

OTHER SUBSTANCES

Extracted: ciprofloxacin

KEY WORDS

serum; rat; ofloxacin is IS

REFERENCE

Rubinstein, E.; Dautrey, S.; Farinoti, R.; St-Julien, L.; Ramon, J.; Carbon, C. Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats. *Antimicrob. Agents Chemother.*, **1995**, *39*, 99–102

SAMPLE

Matrix: blood, saliva, sputum

Sample preparation: 200 μ L Serum, sputum, or saliva + 200 μ L 1.5 μ g/mL pipemidic acid in 100 mM pH 7.0 phosphate buffer + 5 mL dichloromethane, shake for 15 min, centrifuge at 1600 g for 10 min. Remove 4 mL of organic phase and evaporate to dryness under a stream of nitrogen. Dissolve the residue in 200 μ L mobile phase, sonicate for 10 min, inject.

HPLC VARIABLES

Column: 150 \times 6.5 μ m YMC-Pack ODS-AM

Mobile phase: MeCN:10 mM phosphoric acid (pH 7.0) containing 20 mM tetrabutylammonium hydrogen sulfate 1:10

Flow rate: 1.3

Injection volume: 200

Detector: F ex 285 em 460

CHROMATOGRAM

Retention time: 8

Internal standard: pipemidic acid (5)

Limit of quantitation: 10 ng/mL

KEY WORDS

serum

REFERENCE

Koizumi, F.; Ohnishi, A.; Takemura, H.; Okubo, S.; Kagami, T.; Tanaka, T. Effective monitoring of concentrations of ofloxacin in saliva of patients with chronic respiratory tract infections. *Antimicrob. Agents Chemother.*, **1994**, *38*, 1140–1143

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with 30 mM phosphoric acid, centrifuge, inject a 20 μ L aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with 30 mM phosphoric acid, centrifuge, inject a 20 μ L 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 μ L 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 μ L aliquot. Pleural. Dilute human pleural samples with buffer, centrifuge, inject a 20 μ L aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4 .)

HPLC VARIABLES

Column: 200 \times 4.5 μ m Nucleosil C18

Mobile phase: MeOH:MeCN:buffer 13:7:80, adjusted to pH 3.0 with phosphoric acid (Buffer was 15 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide.)

Flow rate: 1

Injection volume: 20-100

Detector: F ex 278 em 446

CHROMATOGRAM

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: ciprofloxacin, norfloxacin

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: blood, urine

Sample preparation: 1 mL Plasma or serum or 200 μ L urine + 1 mL pH 7 buffer + 5 mL dichloromethane, shake, centrifuge at 2500 g for 5 min. Remove 4 mL organic layer and evaporate it under nitrogen, dissolve the residue in 200 μ L mobile phase.

HPLC VARIABLES

Guard column: CT 30/6/4 Resolvosil-BSA-7 (Macherey-Nagel)

Column: 150 \times 4 ET 150/8/4 Resolvosil-BSA-7 (Macherey-Nagel)

Mobile phase: Isopropanol:200 mM pH 8.0 phosphate buffer 3:97

Flow rate: 1

Injection volume: 100

Detector: F ex 298 em 458

CHROMATOGRAM

Retention time: 5.2 (-), 7.5 (+)

Limit of detection: 3 ng/mL (plasma); 80 ng/mL (urine)

KEY WORDS

plasma; serum; chiral

REFERENCE

Lehr, K.-H.; Damm, P. Quantification of the enantiomers of ofloxacin in biological fluids by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *425*, 153-161

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μ L Plasma or serum or 200 μ L urine + 1 mL pH 7 buffer + 2 mL dichloromethane, shake, centrifuge at 2500 g for 5 min. Remove 1 mL organic layer and add it to 1 mL dichloromethane containing 20 μ L diphenylphosphinyl chloride and 20 μ L triethylamine, vortex for 10 s, add 500 μ L L-leucinamide solution, shake for 10 min, extract with 200 μ L 1 M HCl (500 μ L 0.5 M HCl for urine samples), inject 100 μ L of aqueous supernatant. (Prepare L-leucinamide solution by adding 10 mL dichloromethane and 1 mL 5 M NaOH to 500 mg L-leucinamide hydrochloride, shake, discard upper aqueous layer, store dichloromethane layer over anhydrous sodium sulfate.)

HPLC VARIABLES

Column: 125 \times 4.6 5 μ m Nucleosil 120-5C18

Mobile phase: MeCN:200 mM phosphoric acid adjusted to pH 1.85 with tetraethylammonium hydroxide 20:80
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: F ex 298 em 458

CHROMATOGRAM

Retention time: 2.6 (-), 3.8 (+)
Limit of detection: 3 ng/mL (plasma); 80 ng/mL (urine)

KEY WORDS

plasma; serum; derivatization; chiral

REFERENCE

Lehr, K.-H.; Damm, P. Quantification of the enantiomers of ofloxacin in biological fluids by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *425*, 153-161

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 50 μ L 200 μ g/mL desmethylpefloxacin in 10 mM HCl + 1.5 mL 50 mM pH 9 borate buffer containing 50 mM KCl and 21 mM NaOH, vortex for 10 s, add 8 mL dichloromethane, shake for 5 min, centrifuge at 1500 g for 5 min. Remove 7 mL of the organic phase and add it to 200 μ L 100 mM HCl, shake for 5 min, centrifuge at 1500 g, inject a 5 μ L aliquot of the aqueous layer. Urine. Dilute with water 1:40, add 100 μ g/mL desmethylpefloxacin, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:water 10:90 containing 1.36 g/L KH_2PO_4 and 40 mL/L 5 mM aqueous tetrabutylammonium phosphate, pH adjusted to 2.9 with formic acid

Flow rate: 0.7

Injection volume: 5

Detector: F ex 290 em 500

CHROMATOGRAM

Retention time: 8.7

Internal standard: desmethylpefloxacin (10.2)

Limit of detection: 20 ng/mL

KEY WORDS

plasma

REFERENCE

Mignot, A.; Lefebvre, M.A.; Fourtillan, J.B. High-performance liquid chromatographic determination of ofloxacin in plasma and urine. *J.Chromatogr.*, **1988**, *430*, 192-197

SAMPLE

Matrix: blood, vitreous humor

Sample preparation: Serum. 20 μ L Serum + 130 μ L mobile phase, mix, filter, inject a 100 μ L aliquot of the filtrate. Vitreous humor. 15 μ L Vitreous humor + 135 μ L mobile phase, mix, filter, inject a 100 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 220 \times 2.1 5 μ m Nucleosil C18

Mobile phase: MeCN:100 mM pH 3.82 phosphoric acid 75:25

Flow rate: 0.2
Injection volume: 100
Detector: UV 240; UV 280; F ex 280 em 445

CHROMATOGRAM

Limit of detection: 10 ng/mL

KEY WORDS

rabbit; serum; pharmacokinetics

REFERENCE

Perkins, R.J.; Liu, W.; Drusano, G.; Madu, A.; Mayers, M.; Madu, C.; Miller, M.H. Pharmacokinetics of ofloxacin in serum and vitreous humor of albino and pigmented rabbits. *Antimicrob. Agents Chemother.*, **1995**, *39*, 1493-1498

SAMPLE

Matrix: bronchoalveolar lavage fluid

Sample preparation: 1 mL Bronchoalveolar lavage fluid + 20 μ L 30 μ g/mL IS + 1 mL 100 mM pH 7.0 phosphate buffer, vortex, add 5 mL chloroform, shake horizontally at 2 strokes/s 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 40°. Reconstitute residue in 400 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m TSK ODS-80TM (Tosoh)

Mobile phase: THF:0.5% KH₂PO₄ adjusted to pH 2.9 with orthophosphoric acid 1:16

Flow rate: 1

Injection volume: 20

Detector: F ex 290 em 460

CHROMATOGRAM

Retention time: 12

Internal standard: 9-fluoro-2,3-dihydro-3-methyl-10-(1-imidazolyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (8)

Limit of quantitation: 0.5 ng/mL

REFERENCE

Matsubayashi, K.; Une, T.; Osada, Y. Determination of ofloxacin in bronchoalveolar lavage fluid by high-performance liquid chromatography and fluorimetric detection. *J. Chromatogr.*, **1989**, *495*, 354-357

SAMPLE

Matrix: cell lysate

Sample preparation: Sonicate lysed cells for 5 min, filter (0.2 μ m PTFE), add timolol, inject an aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m LiChrospher 100 RP-18

Column: 250 \times 4 5 μ m LiChrospher 100 RP-18

Mobile phase: MeCN:buffer 18:82 (Buffer was 0.5% triethylamine adjusted to pH 2.5 with 78% phosphoric acid.)

Flow rate: 1

Injection volume: 100

Detector: UV 293

CHROMATOGRAM

Retention time: 5.40

Internal standard: timolol (7.70)

Limit of detection: 18 ng/mL

REFERENCE

Fresta, M.; Spadaro, A.; Cerniglia, G.; Roperio, I.M.; Puglisi, G.; Furneri, P.M. Intracellular accumulation of ofloxacin-loaded liposomes in human synovial fibroblasts. *Antimicrob. Agents Chemother.*, **1995**, *39*, 1372–1375

SAMPLE

Matrix: cells

Sample preparation: 100 μ L Cell suspension + 100 μ L cefoperazone solution + 100 μ L Hanks balanced salt solution, sonicate 30 min, add 800 μ L MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μ L mobile phase, inject 75 μ L.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeCN:5 mM pH 2.0 tetrabutylammonium hydrogen sulfate 10:90

Flow rate: 1

Injection volume: 75

Detector: UV 280

CHROMATOGRAM

Retention time: 10.6

Internal standard: ciprofloxacin

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: cells

Sample preparation: Incubate cells in 2 mL 100 mM pH 3.0 glycine-HCl buffer for 2 h at room temperature, centrifuge at 5600 g for 5 min, inject an aliquot.

HPLC VARIABLES

Column: Bondapak C18

Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 25:75

Flow rate: 1.5

Detector: F ex 340 em 425

OTHER SUBSTANCES

Also analyzed: ciprofloxacin, fleroxacin, lomefloxacin, norfloxacin, temafloxacin

REFERENCE

Pascual, A.; Garcia, I.; Conejo, M.C.; Perea, E.J. Fluorometric and high-performance liquid chromatographic measurement of quinolone uptake by human neutrophils. *Eur. J. Clin. Microbiol. Infect. Dis.*, **1991**, *10*, 969–971

SAMPLE

Matrix: formulations

Sample preparation: Dissolve 40 mg freeze-dried nanoparticles in 25 mL acetone:MeOH 90:10 containing a few drops of 100 mM HCl, filter (0.45 μ m), inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Partisil 10-ODS-1 C18**Mobile phase:** MeCN:10 mM KH₂PO₄:triethylamine 14:86:0.2**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 292

OTHER SUBSTANCES**Simultaneous:** pefloxacin (UV 275)

KEY WORDS

nanoparticles

REFERENCE

Fresta, M.; Puglisi, G.; Giammona, G.; Cavallaro, G.; Micali, N.; Furneri, P.M. Pefloxacin mesilate and ofloxacin-loaded polyethylcyanoacrylate nanoparticles: Characterization of the colloidal drug carrier formulation. *J.Pharm.Sci.*, **1995**, *84*, 895–902

SAMPLE**Matrix:** hair

Sample preparation: Wash hair successively with 0.1% sodium dodecyl sulfate and water for 30 min, repeat twice, blot between 2 sheets of paper towel, allow to dry at room temperature. Take a 1 cm fragment of hair, add 500 μL 1 M NaOH, heat at 80° for 30 min, cool, add 500 μL 1 M HCl, add 1 mL 100 mM pH 4.6 potassium hydrogen citrate buffer, add 50 μL 1 μg/mL IS in water. Add the mixture to a Bond-Elut C8 SPE cartridge, elute with 2 mL THF:25 mM orthophosphoric acid 20:80, evaporate eluate to dryness in vacuum, dissolve residue in 150 μL mobile phase, vortex, inject a 60 μL aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 Tosoh 5 μm TSKgel ODS-80Ts**Mobile phase:** MeCN:25 mM orthophosphoric acid adjusted to pH 3.0 with 0.5 M tetra-n-butylamine hydroxide 5:95**Column temperature:** 40**Flow rate:** 1**Injection volume:** 60**Detector:** F ex 295 em 490

CHROMATOGRAM**Retention time:** 8.1

Internal standard: (R)-9-fluoro-2,3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (DS-4632) (10.2) (determine at F ex 280 em 445)

Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES**Extracted:** ciprofloxacin, norfloxacin (determine at F ex 280 em 445)

KEY WORDS

SPE

REFERENCE

Mizuno, A.; Uematsu, T.; Nakashima, M. Simultaneous determination of ofloxacin, norfloxacin and ciprofloxacin in human hair by high-performance liquid chromatography and fluorescence detection. *J.Chromatogr.B*, **1994**, *653*, 187–193

SAMPLE**Matrix:** hair**Sample preparation:** Wash hair several times with 0.1% sodium dodecyl sulfate and with water. Add 500 μL 1 M NaOH to 5-10 cm hair, heat at 80° for 30 min, cool, add 500 μL 1 M HCl, add 1 mL 500 mM pH 7.0 phosphate buffer, add 100 μL 2.5 $\mu\text{g}/\text{mL}$ IS, add 5 mL chloroform, shake mechanically for 20 min, centrifuge at 1670 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES**Guard column:** 15 \times 4.6 TSKguardgel ODS-120T (Tosoh)**Column:** 250 \times 4.6 5 μm TSKgel ODS-120T (Tosoh)**Mobile phase:** MeCN:buffer 18:82 (Buffer was 6.8 g/L KH_2PO_4 adjusted to pH 2.6 with phosphoric acid.)**Flow rate:** 0.8**Injection volume:** 100**Detector:** F ex 290 em 460

CHROMATOGRAM**Retention time:** 10**Internal standard:** 9-fluoro-2,3-dihydro-3-methyl-10-(1-imidazolyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (DL-8357) (8)

KEY WORDS

pharmacokinetics (Ther. Drug Monit. 1995, 17, 101)

REFERENCEMiyazawa, N.; Uematsu, T.; Mizuno, A.; Nagashima, S.; Nakashima, M. Ofloxacin in human hair determined by high performance liquid chromatography. *Forensic Sci. Int.*, **1991**, 51, 65-77

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 \times 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 295

CHROMATOGRAM**Retention time:** 8.8

OTHER SUBSTANCES**Simultaneous:** ciprofloxacin (UV 280), enoxacin (UV 280), feroxacin (UV 280), norfloxacin (UV 280), pipemidic acid (UV 280)

REFERENCEBarbosa, 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:** solutions

HPLC VARIABLES**Guard column:** 20 mm long Supelguard LC-18S (Supelco)**Column:** 250 × 4.6 Suplecasil LC-18S**Mobile phase:** MeCN:buffer:water 10:3.5:86.5 (Buffer was 400 mM tetrabutylammonium hydroxide adjusted to pH 2.85.)**Flow rate:** 1.8**Detector:** UV 280

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:** Prepare a 20 µg/mL solution in MeCN:water 10:90, filter (0.45 µm), inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.5 µm LiChrospher 100 RP-18**Mobile phase:** MeCN:25 mM phosphoric acid 7:93, adjusted to pH 3.09 with 100 mM tetrabutylammonium hydroxide**Flow rate:** 1**Injection volume:** 10**Detector:** UV 295

CHROMATOGRAM**Retention time:** 6.2

OTHER SUBSTANCES**Simultaneous:** ciprofloxacin (UV 280), norfloxacin (UV 280), pipemidic acid (UV 280)

REFERENCEBarbosa, J.; Bergés, R.; Sanz-Nebot, V. Linear solvation energy relationships in reversed-phase liquid chromatography. Prediction of retention of several quinolones. *J.Liq.Chromatogr.*, **1995**, *18*, 3445–3463

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 125 × 4.6 3 µm ODS-Hypersil C18**Mobile phase:** MeOH:THF:670 mM pH 3.0 phosphate buffer 20:0.8:79.2 plus 2 g/L tetrabutylammonium hydrogen sulfate and 2 mL/L 85% phosphoric acid**Flow rate:** 1**Injection volume:** 20**Detector:** UV 286

CHROMATOGRAM**Retention time:** 6.38

OTHER SUBSTANCES

Simultaneous: photodegradation products, fleroxacin

Interfering: ciprofloxacin

REFERENCE

Tiefenbacher, E.-M.; Haen, E.; Przybilla, B.; Kurz, H. Photodegradation of some quinolones used as antimicrobial therapeutics. *J.Pharm.Sci.*, **1994**, *83*, 463–467

SAMPLE

Matrix: tears

Sample preparation: Dry tear sample under nitrogen.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS

Mobile phase: MeCN:phosphoric acid:sodium lauryl sulfate 40:1:0.2:58.8

Flow rate: 1.2

Detector: F ex 358 em 495

CHROMATOGRAM

Retention time: 6.7

Internal standard: triamterene (8.1)

Limit of detection: 10 ng

REFERENCE

Richman, J.; Zolezio, H.; Tang-Liu, D. Comparison of ofloxacin, gentamicin, and tobramycin concentrations in tears and in vitro MICs for 90% of test organisms. *Antimicrob.Agents Chemother.*, **1990**, *34*, 1602–1604

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize a 5 g tissue sample with 100 mL MeCN:0.2% metaphosphoric acid 30:70 at high speed, filter through ca. 2 mm Hyflo Super-Cel coated on a suction funnel. Evaporate filtrate under reduced pressure at 50° to about 30 mL. Apply remaining solution to the SPE cartridge, wash with 20 mL water, elute with 10 mL MeOH. Evaporate eluate to dryness under reduced pressure, dissolve in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Wakosil II 5C18 HG (Wako)

Mobile phase: MeCN:50 mM pH 2.4 phosphate buffer 20:80 containing 2.5 mM 1-heptanesulfonic acid

Flow rate: 0.6

Injection volume: 10

Detector: F ex 295 em 455

CHROMATOGRAM

Retention time: 10

Limit of detection: 10 ng/g

OTHER SUBSTANCES

Extracted: benofloxacin, danofloxacin, enrofloxacin

KEY WORDS

chicken; SPE

REFERENCE

Horie, M.; Saito, K.; Nose, N.; Nakazawa, H. Simultaneous determination of benofloxacin, danofloxacin, enrofloxacin and ofloxacin in chicken tissues by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *653*, 69–76

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 1 mL MeCN, centrifuge at 4000 g for 15 min, inject a 10 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m Hypersil ODS

Mobile phase: MeCN:40 mM phosphoric acid 45:55, final pH 2.56-2.59

Column temperature: 23

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 4.63

Limit of detection: 1 μ g/mL

REFERENCE

Notarianni, L.J.; Jones, R.W. Method for the determination of ofloxacin, a quinolone carboxylic acid antimicrobial, by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *431*, 461–464

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Barbato, F.; Morrica, P.; Seccia, S.; Ventriglia, M. High performance liquid chromatographic analysis of quinolone antibacterial agents. *Farmaco*, **1994**, *49*, 407–410 [simultaneous cinoxacin, ciprofloxacin, flumequine, nalidixic acid, norfloxacin, oxolinic acid, pefloxacin, piromidic acid]

Fabre, D.; Bressolle, F.; Kinowski, J.M.; Bouvet, O.; Paganin, F.; Galtier, M. A reproducible, simple and sensitive HPLC assay for determination of ofloxacin in plasma and lung tissue. Application in pharmacokinetic studies. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1463–1469 [pharmacokinetics; LOQ 5 ng/mL; column temp 50; fluorescence detection]

Lehto, P.; Kivistö, K.T. Effect of sucralfate on absorption of norfloxacin and ofloxacin. *Antimicrob.Agents Chemother.*, **1994**, *38*, 248–251 [extracted norfloxacin; plasma; urine; LOQ 100 ng/mL; pharmacokinetics]

Lyon, D.J.; Cheung, S.W.; Chan, C.Y.; Cheng, A.F. Rapid HPLC assay of cinafloxacin, feroxacin, levofloxacin, sparfloxacin and tosufloxacin. *J.Antimicrob.Chemother.*, **1994**, *34*, 446–448

Mueller, B.A.; Brierton, D.G.; Abel, S.R.; Bowman, L. Effect of feeding with Ensure on oral bioavailabilities of ofloxacin and ciprofloxacin. *Antimicrob.Agents Chemother.*, **1994**, *38*, 2101–2105 [extracted ciprofloxacin; plasma; ultrafiltration; fluorescence detection; A-57084 (IS); LOQ 9.5 ng/mL; pharmacokinetics]

Ohkubo, T.; Kudo, M.; Sugawara, K.; Sawada, Y. The determination of ofloxacin in human skin tissue by high-performance liquid chromatography and correlation between skin tissue concentration and serum level of ofloxacin. *J.Pharm.Pharmacol.*, **1994**, *46*, 522–524

Sánchez Navarro, A.; Martínez Cabarga, M.; Dominguez-Gil Hurlé, A. Oral absorption of ofloxacin administered together with aluminum. *Antimicrob.Agents Chemother.*, **1994**, *38*, 2510–2512 [plasma; ciprofloxacin (IS); LOD 50 ng/mL; pharmacokinetics]

Zeng, S.; Zhang, L.; Liu, Z.Q. [Quantification of the enantiomers of ofloxacin in human urine by RP-HPLC with chiral mobile phase additive]. *Yao Hsueh Hsueh Pao*, **1994**, *29*, 223–227

Israel, D.; Gillum, G.; Turik, M.; Harvey, K.; Ford, J.; Dalton, H.; Towle, M.; Echols, R.; Heller, A.H.; Polk, R. Pharmacokinetics and serum bactericidal titers of ciprofloxacin and ofloxacin following mul-

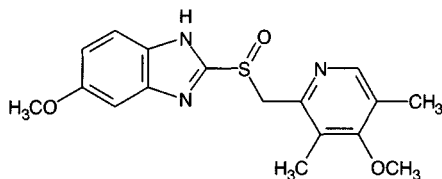
- multiple oral doses in healthy volunteers. *Antimicrob. Agents Chemother.*, **1993**, *37*, 2193–2199 [pharmacokinetics; serum; extracted ciprofloxacin; norfloxacin (IS)]
- Ohkubo, T.; Kudo, M.; Sugawara, K. Determination of ofloxacin in human serum by high-performance liquid chromatography with column switching. *J. Chromatogr.*, **1992**, *573*, 289–293
- Budvári-Bárány, Z.; Szász, G.; Takács-Novák, K.; Hermecz, I.; Lore, A. The pH influence on the HPLC-retention of chemotherapeutic fluoroquinolone derivatives. *J. Liq. Chromatogr.*, **1991**, *14*, 3411–3424 [also amifloxacin, ciprofloxacin, lomefloxacin, nalidixic acid, norfloxacin, oxolinic acid, pefloxacin]
- Le Coguic, A.; Bidault, R.; Farinotti, R.; Dauphin, A. Determination of ofloxacin in plasma and urine by liquid chromatography. *J. Chromatogr.*, **1988**, *434*, 320–323 [fluorescence detection; LOD 0.5 ng/mL]
- Carlucci, G.; Guadagni, S.; Palumbo, G. Determination of ofloxacin, a new oxazine derivative in human serum, urine, and bile by high-performance liquid chromatography. *J. Liq. Chromatogr.*, **1986**, *9*, 2539–2547 [norfloxacin (IS)]
- Groeneveld, A.J.; Brouwers, J.R. Quantitative determination of ofloxacin, ciprofloxacin, norfloxacin and pefloxacin in serum by high pressure liquid chromatography. *Pharm. Weekbl. [Sci.]*, **1986**, *8*, 79–84

Omeprazole

Molecular formula: C₁₇H₁₉N₃O₃S

Molecular weight: 345.4

CAS Registry No.: 73590-58-6



SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 1 µg IS + 5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18

Mobile phase: MeOH:water 50:50 containing 1% triethylamine, pH adjusted to 7.4 with phosphoric acid

Flow rate: 2

Injection volume: 100

Detector: UV 302

CHROMATOGRAM

Retention time: 5.3

Internal standard: 5-methyl-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (H168/24) (8.4)

Limit of quantitation: 50 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Balian, J.D.; Sukhova, N.; Harris, J.W.; Hewett, J.; Pickle, L.; Goldstein, J.A.; Woosley, R.L.; Flockhart, D.A. The hydroxylation of omeprazole correlates with S-mephenytoin metabolism: A population study. *Clin.Pharmacol.Ther.*, **1995**, *57*, 662-669

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg C2 SPE cartridge (Varian) with 1 mL MeOH and 1 mL water. 1 mL Plasma + 50 µL 5 µg/mL IS, add to SPE cartridge, apply a vacuum so the sample moves through at 0.5 mL/min, wash with 1 mL water, dry under vacuum for 10 min, elute with two 1 mL portions of MeCN (do not allow to run dry). Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 µL MeOH, vortex for 2 min, add 200 µL pH 9.3 carbonate buffer, inject a 100-190 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.5 µm Resolvosil BSA-7 bovine serum albumin (Macherey-Nagel)

Mobile phase: n-Propanol:50 mM pH 7.0 ammonium phosphate buffer 0.75:99.25 (n-Propanol concentration should be optimized for each column over the range 0.05-1%).

Flow rate: 1.5

Injection volume: 100-190

Detector: UV 302

CHROMATOGRAM

Retention time: 13.88 (+), 18.27 (-)

Internal standard: 2-[(4-methoxy-2-pyridinyl)methyl]sulfinyl)-4,6-dimethyl-1H-benzimidazole (Astra, Hassle AB, Sweden) (9.73)

Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; chiral; protect from light; SPE

REFERENCE

Cairns, A.M.; Chiou, R.H.-Y.; Rogers, J.D.; Demetriades, J.L. Enantioselective high-performance liquid chromatographic determination of omeprazole in human plasma. *J.Chromatogr.B*, **1995**, *666*, 323-328

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 150 μ L water + 5 mL diethyl ether:dichloromethane 70:30, vortex for 5 min, centrifuge at 6° at 300-850 g for 10 min. Remove 4 mL of the organic layer and evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 500 μ L buffer, refrigerate until injection, inject a 100 μ L aliquot. (Buffer was MeCN:water 35:65 containing 1 mL/L n-octylamine and 5 mM N-acetohydroxamic acid, pH adjusted to 7.5 with 85% phosphoric acid.)

HPLC VARIABLES

Column: 150 or 250 \times 4.6 5 μ m Hi-Chrom Reversible octadecylsilane (Regis)

Mobile phase: MeCN:water 35:65 containing 1 mL/L n-octylamine and 5 mM N-acetohydroxamic acid, pH adjusted to 7.0 with 85% phosphoric acid

Column temperature: 40-43

Flow rate: 1 for 15 min then 2.5

Injection volume: 100

Detector: UV 285

CHROMATOGRAM

Retention time: 7.6

Internal standard: omeprazole

OTHER SUBSTANCES

Extracted: lansoprazole

KEY WORDS

plasma; omeprazole is IS; omeprazole has been determined using this assay, LOQ 10 ng/mL (Antimicrob. Agents Chemother. 1995, 39, 2078)

REFERENCE

Karol, M.D.; Granneman, G.R.; Alexander, K. Determination of lansoprazole and five metabolites in plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *668*, 182-186

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L MeOH:75 mM pH 9.3 carbonate buffer 20:80 + 5 mL dichloromethane:MeCN 80:20, mix for 10 min, centrifuge at 2000 g for 5

min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Nucleosil 5C18

Mobile phase: MeCN:20 mM pH 7.5 phosphate buffer 37:63

Column temperature: 40

Flow rate: 1

Injection volume: 100

Detector: UV 302

CHROMATOGRAM

Limit of quantitation: 5 ng/mL

KEY WORDS

rat; plasma; pharmacokinetics

REFERENCE

Katashima, M.; Yamamoto, K.; Sugiura, M.; Sawada, Y.; Iga, T. Comparative pharmacokinetic/pharmacodynamic study of proton pump inhibitors, omeprazole and lansoprazole in rats. *Drug Metab. Dispos.*, **1995**, *23*, 718–723

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 \times 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 303

CHROMATOGRAM

Retention time: 3.72

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazox-

ide, diclofenac, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, sulfinpyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: acebutolol, bromazepam, carbamazepine, dihydralazine, nadolol, nalbuphine, procainamide, procarbazine, strychnine

KEY WORDS

whole blood; plasma

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 + 500 ng IS + 6 mL dichloromethane, extract, centrifuge. Remove organic layer and evaporate it, dissolve the residue in 100 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 120 mm long 5 μ m Hypersil ODS

Mobile phase: MeOH: water: triethylamine 55:44:1, pH 7

Flow rate: 0.8

Detector: UV 302

CHROMATOGRAM

Retention time: 7.2

Internal standard: 5-methyl-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (H 168/24) (11.0)

Limit of quantitation: 10 ng/mL

KEY WORDS

plasma

REFERENCE

Treiber, G.; Walker, S.; Klotz, U. Omeprazole-induced increase in the absorption of bismuth from tri-potassium dicitrato bismuthate. *Clin. Pharmacol. Ther.*, **1994**, *55*, 486–491

SAMPLE**Matrix:** blood**Sample preparation:** Condition a Bond Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Add 20 μ L plasma + 40 ng nitrazepam to SPE cartridge, wash with 2 mL water, elute with 250 μ L MeOH, inject a 60 μ L aliquot of the eluate.

HPLC VARIABLES**Column:** 125 \times 4 μ m LiChro CART C18**Mobile phase:** MeCN:20 mM pH 7.4 Na₂HPO₄ 35:65**Flow rate:** 1**Injection volume:** 60**Detector:** UV 310

CHROMATOGRAM**Internal standard:** nitrazepam**Limit of detection:** 25 ng/mL

KEY WORDSplasma; rat; SPE; pharmacokinetics

REFERENCEWatanabe, K.; Furuno, K.; Eto, K.; Oishi, R.; Gomita, Y. First-pass metabolism of omeprazole in rats. *J.Pharm.Sci.*, **1994**, *83*, 1131-1134

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 100 μ L 1 M NaH₂PO₄ + 100 μ L 50-100 μ g/mL IS in MeOH:pH 9.3 carbonate buffer (I = 0.1) 20:80, mix, add 1 mL dichloromethane, shake for 10 min, centrifuge twice at 2500 g for 10 min, inject a 150 μ L aliquot of the organic layer.

HPLC VARIABLES**Column:** 150 \times 4.5 μ m LiChrosorb Si 60**Mobile phase:** Dichloromethane:MeOH containing 5% concentrated ammonium hydroxide 96.5:3.5**Flow rate:** 1.5**Injection volume:** 150**Detector:** UV 302

CHROMATOGRAM**Retention time:** 4**Internal standard:** 4,6-dimethyl-2-[[[(4-methoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (H153/52) (5.5)**Limit of detection:** 20 nM (plasma)

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDSplasma; normal phase

REFERENCELagerström, P.-O.; Persson, B.-A. Determination of omeprazole and metabolites in plasma and urine by liquid chromatography. *J.Chromatogr.*, **1984**, *309*, 347-356

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 1 mL Plasma + 100 μ L 100 μ g/mL phenacetin in MeOH + 5 mL dichloromethane + 250 mg NaCl + 500 μ L 0.5 M pH 8.0 phosphate buffer, vortex for 10 min, centrifuge at 1610 g for 30 min. Remove the organic phase and evaporate it under vacuum at 40°, reconstitute the residue with 300 μ L mobile phase, filter (0.45 μ m), inject a 30 μ L aliquot. Urine. 1 mL Urine + 100 μ L 200 μ g/mL phenacetin in MeOH + 5 mL dichloromethane + 250 mg NaCl + 500 μ L 0.5 M pH 8.0 phosphate buffer, vortex for 10 min, centrifuge at 1610 g for 30 min. Remove the organic phase and evaporate it under vacuum at 40°, reconstitute the residue with 200 μ L mobile phase, inject a 10-30 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Capcell Pak C18 SG 120 (Shiseido)**Mobile phase:** MeCN:50 mM pH 8.5 phosphate buffer 25:75**Flow rate:** 0.8**Injection volume:** 10-30**Detector:** UV 302

CHROMATOGRAM**Retention time:** 16.8**Internal standard:** phenacetin (13.6)**Limit of quantitation:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites**Noninterfering:** cimetidine, ranitidine, famotidine

KEY WORDS

plasma; pharmacokinetics

REFERENCEKobayashi, K.; Chiba, K.; Sohn, D.-R.; Kato, Y.; Ishizaki, T. Simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkaline-resistant polymer-coated C18 column. *J.Chromatogr.*, **1992**, *579*, 299-305

SAMPLE**Matrix:** blood, urine**Sample preparation:** 1 mL Plasma or urine + 100 μ L 1 M NaH₂PO₄ + 100 μ L 50-100 μ g/mL IS in MeOH:pH 9.3 carbonate buffer (I = 0.1) 20:80, mix, add 10 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 500 μ L MeCN:pH 7.5 phosphate buffer (I=0.05), inject a 150 μ L aliquot.

HPLC VARIABLES**Guard column:** 30 \times 4.6 Spheri-5 RP-8**Column:** 150 \times 4.5 5 μ m Polygosil C18 (Macherey-Nagel)**Mobile phase:** MeCN:pH 7.5 phosphate buffer (I=0.05) 30:70**Flow rate:** 1**Injection volume:** 150**Detector:** UV 302

CHROMATOGRAM**Retention time:** 7.5

Internal standard: 5-methyl-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (H168/24) (10)

Limit of quantitation: 25 nM (plasma); 50 nM (urine)

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: cimetidine, ranitidine

KEY WORDS

plasma

REFERENCE

Lagerström, P.-O.; Persson, B.-A. Determination of omeprazole and metabolites in plasma and urine by liquid chromatography. *J.Chromatogr.*, **1984**, *309*, 347-356

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Incubation mixture + 2 mL dichloromethane:butanol 99:1, cool on ice, add 100 μ L 1 M NaH_2PO_4 + IS, vortex for 1 min, centrifuge at 1000 g for 5 min. Remove 1.5 mL of the organic phase and evaporate it to dryness under nitrogen, reconstitute in 150 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3 7 μ m Brownlee Aquapore silica

Column: 125 \times 4 4 μ m Superspher SI-60

Mobile phase: Dichloromethane:5% ammonium hydroxide in MeOH:isopropanol 191:8:1

Flow rate: 1.5

Injection volume: 50

Detector: UV 302

CHROMATOGRAM

Retention time: 2.5

Internal standard: 4,6-Dimethyl-2-[[[(4-methoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (3.3)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

normal phase

REFERENCE

Andersson, T.; Lagerstrom, P.-O.; Miners, J.O.; Veronese, M.E.; Weidolf, L.; Birkett, D.J. High-performance liquid chromatographic assay for human liver microsomal omeprazole metabolism. *J.Chromatogr.*, **1993**, *619*, 291-297

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in EtOH, inject a 10-20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Chiralpak AD

Mobile phase: Hexane:EtOH 80:20

Column temperature: 35

Flow rate: 1

Injection volume: 10-20

Detector: UV 302

CHROMATOGRAM

Retention time: k' 4.86 (of first (-) enantiomer)

OTHER SUBSTANCES

Simultaneous: lansoprazole, pantoprazole, timoprazole

KEY WORDS

chiral; $\alpha = 1.81$

REFERENCE

Balmér, K.; Persson, B.-A.; Lagerström, P.-O. Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases. *J.Chromatogr.A*, **1994**, 660, 269-273

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Yasuda, S.; Horai, Y.; Tomono, Y.; Nakai, H.; Yamato, C.; Manabe, K.; Kobayashi, K.; Chiba, K.; Ishizaki, T. Comparison of the kinetic disposition and metabolism of E3810, a new proton pump inhibitor, and omeprazole in relation to S-mephenytoin 4'-hydroxylation status. *Clin.Pharmacol.Ther.*, **1995**, 58, 143-154 [LOQ 5 ng/mL; pharmacokinetics]

Kobayashi, K.; Chiba, K.; Tani, M.; Kuroiwa, Y.; Ishizaki, T. Development and preliminary application of a high-performance liquid chromatographic assay for omeprazole metabolism in human liver microsomes. *J.Pharm.Biomed.Anal.*, **1994**, 12, 839-844

Arvidsson, T.; Collijn, E.; Tivert, A.M.; Rosen, L. Peak distortion in the column liquid chromatographic determination of omeprazole dissolved in borax buffer. *J.Chromatogr.*, **1991**, 586, 271-276

Erlandsson, P.; Isaksson, R.; Lorentzon, P.; Lindberg, P. Resolution of the enantiomers of omeprazole and some of its analogues by liquid chromatography on a trisphenylcarbamoylcellulose-based stationary phase. The effect of the enantiomers of omeprazole on gastric glands. *J.Chromatogr.*, **1990**, 532, 305-319

Amantea, M.A.; Narang, P.K. Improved procedure for quantitation of omeprazole and metabolites using reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1988**, 426, 216-222

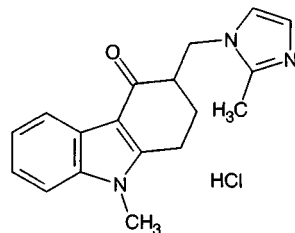
Mihaly, G.W.; Prichard, P.J.; Smallwood, R.A.; Yeomans, N.D.; Louis, W.J. Simultaneous high-performance liquid chromatographic analysis of omeprazole and its sulphone and sulphide metabolites in human plasma and urine. *J.Chromatogr.*, **1983**, 278, 311-319

Ondansetron

Molecular formula: C₁₈H₁₉N₃O

Molecular weight: 293.4

CAS Registry No.: 99614-02-5 (ondansetron), 116002-70-1 (ondansetron), 99614-01-4 (ondansetron hydrochloride dihydrate), 103639-04-9 (ondansetron hydrochloride dihydrate)



SAMPLE

Matrix: beverages

Sample preparation: Juice, soft drinks. Inject a 10 μ L aliquot. Tea. Cool 60 mL tea in an ice bath to about 0° in 1 min. Remove a 10 mL aliquot, add 1 drop concentrated HCl, add 10 mL MeCN, mix, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 5 μ m C18 (Phase Separations)

Column: 250 \times 4.5 5 μ m Spherisorb cyanopropyl

Mobile phase: MeCN:20 mM pH 5.4 phosphate buffer 60:40

Flow rate: 2

Injection volume: 10

Detector: UV 216

CHROMATOGRAM

Retention time: 6.5

KEY WORDS

tea; apple juice; soft drinks; stability indicating

REFERENCE

Yamreudeewong, W.; Danthi, S.N.; Hill, R.A.; Fox, J.L. Stability of ondansetron hydrochloride injection in various beverages. *Am.J.Health-Syst.Pharm.*, **1995**, 52, 2011–2014

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg cyanopropyl SPE cartridge (J & W Scientific) with 2 volumes of MeOH and 2 volumes of water, do not allow to dry. 1 mL Serum + 30 μ L 10 μ g/mL prazosin in EtOH, vortex for 10 s, add to SPE cartridge, wash with 1 volume MeOH:water 10:90, dry under full vacuum for 20 min, elute with four 250 μ L aliquots of MeOH:triethylamine 999:1. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, dissolve the residue in 250 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: 500 \times 4.6 10 μ m Chiralcel OD precolumn (sic)

Column: 250 \times 4.6 10 μ m Chiralcel OD

Mobile phase: Hexane:95% EtOH:isopropanol:MeCN 65:25:10:1

Flow rate: 1

Injection volume: 100

Detector: UV 216

CHROMATOGRAM

Retention time: 9.96 (R-(-)), 11.56 (S-(+))

Internal standard: prazosin (7.99)

Limit of detection: 2.5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

serum; SPE; chiral

REFERENCE

Kelly, J.W.; He, L.; Stewart, J.T. High-performance liquid chromatographic separation of ondansetron enantiomers in serum using a cellulose-derivatized stationary phase and solid-phase extraction. *J.Chromatogr.*, **1993**, 622, 291–295

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Bond Elut CN SPE cartridge with two 1 mL portions of MeOH, two 1 mL portions of isopropanol:ammonia 99:1, and two 1 mL portions of water, do not allow to dry. Add 50 μ L 0.5 M HCl then 1 mL plasma to the SPE cartridge, dry the cartridge with a vacuum, wash with two 1 mL portions of water, allow to dry, wash with two 1 mL portions of MeCN, allow to dry, elute with two 1 mL portions of isopropanol:ammonia 99:1, evaporate the eluate at 45° under vacuum, reconstitute in 200 μ L mobile phase, sonicate for 5 min, vortex, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Spherisorb S3W silica

Mobile phase: MeCN:buffer 40:60 (Buffer was 25 mM sodium acetate adjusted to pH 4.2 with glacial acetic acid.)

Column temperature: 35

Flow rate: 1

Injection volume: 100

Detector: UV 305

CHROMATOGRAM

Retention time: 4-5

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites, fluorouracil, melphalan

Noninterfering: cisplatin

KEY WORDS

SPE; plasma

REFERENCE

Colthup, P.V.; Felgate, C.C.; Palmer, J.L.; Scully, N.L. Determination of ondansetron pharmacokinetics in the young and elderly. *J.Pharm.Sci.*, **1991**, 80, 868–871

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Bondelut CN SPE cartridge with 2 mL MeOH, 2 mL isopropanol:ammonia 99:1, and 2 mL water. Mix 1 mL plasma with 50 μ L 0.5 M HCl and add to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeCN, elute with 2 mL isopropanol:ammonia 99:1, evaporate the eluate, dissolve the residue in 200 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Spherisorb S3W silica

Mobile phase: MeCN:25 mM pH 4.2 sodium acetate 40:60

Column temperature: 35

Injection volume: 100

Detector: UV 305

CHROMATOGRAM

Limit of quantitation: 1 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Colthup, P.V.; Palmer, J.L. The determination in plasma and pharmacokinetics of ondansetron. *Eur.J.Cancer Clin.Oncol.*, **1989**, 25 Suppl 1, S71-S74

SAMPLE

Matrix: formulations

Sample preparation: Dilute with mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: ODS

Column: 250 \times 4.6 5 μ m 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: 11.6

Limit of detection: 70 ng/mL

OTHER SUBSTANCES

Simultaneous: diphenhydramine, methyl paraben, 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

Sample preparation: Dilute with an equal volume of saline (if necessary), filter (0.2 μ m), inject a 50 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Accubond CN (J & W)

Mobile phase: MeCN:buffer 60:40 adjusted to pH 6.0 with 1 M NaOH (Buffer was 20 mM KH_2PO_4 containing 5 mM octanesulfonic acid.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 305

CHROMATOGRAM

Retention time: 8-9

KEY WORDS

saline; injections; stability-indicating

REFERENCE

Chung, K.C.; Moon, Y.S.K.; Chin, A.; Ulrich, R.W.; Gill, M.A. Compatibility of ondansetron hydrochloride and piperacillin sodium tazobactam sodium during simulated Y-site administration. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1554–1556

SAMPLE

Matrix: formulations

Sample preparation: Filter (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 \times 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: 3.3

OTHER SUBSTANCES

Simultaneous: cyclophosphamide

KEY WORDS

injections; saline; 5% dextrose

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: Filter (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 \times 4.6 5 μm Spherisorb nitrile

Mobile phase: MeCN:20 mM pH 5.4 phosphate buffer 60:40

Flow rate: 1.2

Injection volume: 20

Detector: UV 216

CHROMATOGRAM

Retention time: 9.3

OTHER SUBSTANCES

Noninterfering: cyclophosphamide

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 2 volumes water (if necessary), filter (0.45 μm), inject an aliquot.

HPLC VARIABLES**Column:** 220 \times 4.6 10 μm Spheri-10**Mobile phase:** MeCN:DMF:pH 6.5 sodium acetate 15:4:85**Flow rate:** 1**Detector:** UV 305

KEY WORDS

total parental nutrient; injections; stability-indicating

REFERENCEKirkham, J.C.; Rutherford, E.T.; Cunningham, G.N.; Daneshmand, K.A.; Falls, A.L. Stability of ondansetron hydrochloride in a total parenteral nutrient admixture. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1557-1558

SAMPLE**Matrix:** formulations**Sample preparation:** Inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 100 \times 4.6 Spheri-5 RP-8**Mobile phase:** MeCN:buffer 45:55 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 1 M KOH.)**Flow rate:** 1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5.9**Limit of detection:** 90 ng/mL

OTHER SUBSTANCES**Simultaneous:** glycopyrrolate**Noninterfering:** degradation products

KEY WORDS

injections; saline

REFERENCEVenkateshwaran, T.G.; King, D.T.; Stewart, J.T. HPLC determination of ondansetron-atropine and ondansetron-glycopyrrolate mixtures in 0.9% sodium chloride injection. *J.Liq.Chromatogr.*, **1995**, *18*, 2647-2659

SAMPLE**Matrix:** formulations

HPLC VARIABLES**Column:** 250 \times 4.6 5 μm Zorbax Rx-C8 base deactivated octylsilane (Mac-Mod Analytical)**Mobile phase:** MeCN:10 mM KH_2PO_4 adjusted to pH 4.0 with 1 M KOH 23:77**Flow rate:** 1

Injection volume: 20

Detector: UV 273

CHROMATOGRAM

Retention time: 14.8

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: metoclopramide

KEY WORDS

saline; injections

REFERENCE

Venkateshwaran, T.G.; King, D.T.; Stewart, J.T. HPLC determination of a metoclopramide and ondansetron mixture in 0.9% sodium chloride injection. *J.Liq.Chromatogr.*, **1995**, *18*, 117–126

SAMPLE

Matrix: formulations

Sample preparation: 1 mL Sample + 50 μ L 150 ng/mL cimetidine, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.8 Spherisorb S5CN

Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH_2PO_4 adjusted to pH 5.4 with 1 M NaOH.)

Flow rate: 1.5

Injection volume: 10

Detector: UV 216

CHROMATOGRAM

Retention time: 9.5

Internal standard: cimetidine (4.5)

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 1:2, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Accubond CN (J & W)

Mobile phase: MeCN:20 mM KH_2PO_4 and 5 mM octanesulfonic acid 50:50, pH adjusted to 6.0 with 1 M NaOH

Flow rate: 1.5

Injection volume: 50

Detector: UV 305

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Noninterfering: ranitidine, paclitaxel

KEY WORDS

injections; 5% dextrose; stability-indicating

REFERENCE

Burm, J.-P.; Jhee, S.S.; Chin, A.; Moon, Y.S.K.; Jeong, E.; Nii, L.; Fox, J.L.; Gill, M.A. Stability of paclitaxel with ondansetron hydrochloride or ranitidine hydrochloride during simulated Y-site administration. *Am.J.Hosp.Pharm.*, **1994**, *51*, 1201-1204

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl

Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH₂PO₄ adjusted to pH 5.4 with 1 M NaOH.)

Flow rate: 1

Injection volume: 20

Detector: UV 233

CHROMATOGRAM

Retention time: 10.5

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, doxorubicin, methyl paraben, vincristine

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 1:10, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 5 μm Spherisorb CN

Mobile phase: MeCN:20 mM KH₂PO₄ 50:50, pH adjusted to 5.40 with 1 M NaOH

Flow rate: 1.5

Injection volume: 10

Detector: UV 216

CHROMATOGRAM

Retention time: 8.8-10.0

OTHER SUBSTANCES

Simultaneous: hydromorphone, morphine

KEY WORDS

injections; saline; stability-indicating

REFERENCE

Trissel, L.A.; Xu, Q.; Martinez, J.F.; Fox, J.L. Compatibility and stability of ondansetron hydrochloride with morphine sulfate and with hydromorphone hydrochloride in 0.9% sodium chloride injection at 4, 22, and 32°C. *Am.J.Hosp.Pharm.*, **1994**, *51*, 2138–2142

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 220 × 4.6 5 μm silica (Brownlee)

Mobile phase: MeCN:6.25 mM NaH₂PO₄ adjusted to pH 3.0 with concentrated phosphoric acid 40:60

Flow rate: 1

Injection volume: 50

Detector: UV 216

CHROMATOGRAM

Retention time: 8.6

Limit of detection: 8 ng/mL

OTHER SUBSTANCES

Simultaneous: dacarbazine, doxorubicin

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: microsomal incubations

Sample preparation: 750 μL Microsomal incubation + 1.5 mL MeCN, concentrate, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil C8 BDS

Mobile phase: MeCN:40 mM pH 4.5 ammonium acetate 20:80

Flow rate: 0.8

Detector: Radioactivity

CHROMATOGRAM

Retention time: 26

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver; ¹⁴C labeled

REFERENCE

Dixon, C.M.; Colthup, P.V.; Serabjit-Singh, C.J.; Kerr, B.M.; Boehlert, C.C.; Park, G.R.; Tarbit, M.H. Multiple forms of cytochrome P450 are involved in the metabolism of ondansetron in humans. *Drug Metab.Dispos.*, **1995**, *23*, 1225–1230

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 10 μm Partisil ODS1**Mobile phase:** MeOH:50 mM pH 3.0 phosphoric acid 40:60**Column temperature:** 30**Flow rate:** 1.5**Detector:** Radioactivity

KEY WORDS¹⁴C labeled

REFERENCE

Collett, A.; Sims, E.; Walker, D.; He, Y.-L.; Ayrton, J.; Rowland, M.; Warhurst, G. Comparison of HT29-18-C₁ and Caco-2 cell lines as models for studying intestinal paracellular drug absorption. *Pharm.Res.*, **1996**, *13*, 216–221

SAMPLE**Matrix:** solutions**Sample preparation:** Inject an aliquot of an aqueous solution.

HPLC VARIABLES**Column:** 250 × 4 Nucleosil C18**Mobile phase:** MeOH:THF:buffer 30:5:65 (Buffer was 100 mM triethylamine adjusted to pH 3.0 with nitric acid.)**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 7.40

OTHER SUBSTANCES**Simultaneous:** granisetron, tropisetron

REFERENCE

Barbato, F.; Immacolata La Rotonda, M.; Quaglia, F. Retention behaviour of anti-emetic serotonin antagonists in reversed phase high performance liquid chromatography. *Farmaco*, **1995**, *50*, 875–880

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 5 μm YMC-Pack ODS-A**Mobile phase:** MeCN:MeOH:isopropanol:20 mM NaH₂PO₄ 50:15:5:30**Flow rate:** 1**Detector:** UV 216

CHROMATOGRAM**Retention time:** 3.7

OTHER SUBSTANCES**Simultaneous:** impurities

REFERENCE

- Ong, C.P.; Chow, K.K.; Ng, C.L.; Ong, F.M.; Lee, H.K.; Li, S.F.Y. Use of overlapping resolution mapping scheme for optimization of the high-performance liquid chromatographic separation of pharmaceuticals. *J.Chromatogr.A*, **1995**, 692, 207–212
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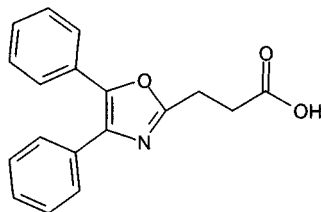
- Fischer, V.; Vickers, A.E.M.; Heitz, F.; Mahadevan, S.; Baldeck, J.-P.; Minery, P.; Tynes, R. The polymorphic cytochrome P-4502D6 is involved in the metabolism of both 5-hydroxytryptamine antagonists, tropisetron and ondansetron. *Drug Metab.Dispos.*, **1994**, 22, 269–274 [microsomal incubations; human; liver; extracted metabolites; gradient; column temp 40; radioactivity detection]
- Pompilio, F.M.; Fox, J.L.; Inagaki, K.; Burm, J.-P.; Jhee, S.; Gill, M.A. Stability of ranitidine hydrochloride with ondansetron hydrochloride or fluconazole during simulated Y-site administration. *Am.J.Hosp.Pharm.*, **1994**, 51, 391–394 [stability-indicating; injections; 5% dextrose]
- Williams, C.L.; Sanders, P.L.; Laizure, S.C.; Stevens, R.C.; Fox, J.L.; Hak, L.J. Stability of ondansetron hydrochloride in syrups compounded from tablets. *Am.J.Hosp.Pharm.*, **1994**, 51, 806–809 [stability-indicating; syrup]
- Graham, C.L.; Dukes, G.E.; Kao, C.-F.; Bertsch, J.M.; Hak, L.J. Stability of ondansetron in large-volume parenteral solutions. *Ann.Pharmacother.*, **1992**, 26, 768–771 [injections; saline; 5% dextrose; lactated Ringer's solution; simultaneous degradation products]

Oxaprozin

Molecular formula: C₁₆H₁₅NO₃

Molecular weight: 293.3

CAS Registry No.: 21256-18-8



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 µg ketoprofen + 200 µL 1 M HCl + 4-5 mL ethyl acetate, vortex for 1.5-2 min, centrifuge at 400 g for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 40-50°, reconstitute the residue in 200 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 100 × 5 10 µm spherical C18 radial compression (Waters)

Mobile phase: MeCN:water 45:55 containing 2.5 mL/L acetic acid

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 8

Internal standard: ketoprofen (5)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, indomethacin, phenylbutazone, salicylic acid

Noninterfering: ibuprofen, piroxicam

Interfering: fenoprofen, flurbiprofen

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Matlis, R.; Greenblatt, D.J. Rapid high-performance liquid chromatographic analysis of oxaprozin, a non-steroidal anti-inflammatory agent. *J.Chromatogr.*, **1984**, *310*, 445-449

SAMPLE

Matrix: blood

Sample preparation: 500 µL Plasma + 2.5 mL water + 1 mL 20-60 µg/mL ketoprofen in 100 mM pH 4.8 acetate buffer, adjust to pH 2 with about 200 µL 1 M HCl, add 8 mL ether, shake mechanically for 10 min, centrifuge at 2000 rpm for 5 min. Remove 7 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 500 µL mobile phase, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 µm Chromegabond C18 (E.S. Industries)

Mobile phase: MeCN:buffer 62:38 (Buffer was 50 mM NaH₂PO₄ adjusted to pH 3.9 with phosphoric acid.)

Flow rate: 1.5

Injection volume: 10

Detector: UV 290

CHROMATOGRAM**Retention time:** 5.5**Internal standard:** ketoprofen (3.5)**Limit of detection:** 500 ng/mL

KEY WORDS

plasma; human; pharmacokinetics

REFERENCE

McHugh, S.L.; Kirkman, S.K.; Knowles, J.A. Macro- and micromethods for high-performance liquid chromatographic analysis of oxaprozín in plasma. *J.Pharm.Sci.*, **1980**, *69*, 794–796

SAMPLE**Matrix:** blood, urine

Sample preparation: 50 μ L Plasma or urine + 50 μ L 100 mM pH 7 phosphate buffer + 50 μ L 5-20 μ g/mL ketoprofen in 100 mM pH 7 phosphate buffer + 200 μ L ether, vortex for 5 s, centrifuge, repeat extraction twice. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES**Guard column:** 50 \times 2.1 37-45 μ m Co:Pell ODS**Column:** 300 \times 4.6 10 μ m Chromegabond C18 (E.S. Industries)**Mobile phase:** MeCN:buffer 60:40 (Buffer was 50 mM NaH₂PO₄ adjusted to pH 3.9 with phosphoric acid.)**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 4.5**Internal standard:** ketoprofen (3.5)**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

McHugh, S.L.; Kirkman, S.K.; Knowles, J.A. Macro- and micromethods for high-performance liquid chromatographic analysis of oxaprozín in plasma. *J.Pharm.Sci.*, **1980**, *69*, 794–796

SAMPLE**Matrix:** bulk

Sample preparation: Prepare a 400 μ g/mL solution in mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 μ Bondapak C18**Mobile phase:** MeCN:MeOH:buffer 25:25:50, adjusted to pH 4.2 with 85% phosphoric acid (Buffer was 10 mM KH₂PO₄ containing 5 mM sodium 1-decanesulfonate.)**Flow rate:** 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 19.8

Limit of detection: 54 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

stability-indicating

REFERENCE

Ibrahim, F.B. Quantitative determination of oxaprozin and several of its related compounds by high-performance reversed-phase liquid chromatography. *J.Liq.Chromatogr.*, **1995**, *18*, 2621–2633

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 2.1 C-8 (Brownlee)

Mobile phase: MeCN:buffer 28:72 (Buffer was 500 mM pH 4.6 sodium phosphate buffer containing 5 mM tetrabutylammonium phosphate.)

Flow rate: 0.5

Detector: UV 254

CHROMATOGRAM

Retention time: 15.71

OTHER SUBSTANCES

Simultaneous: metabolites, glucuronides

REFERENCE

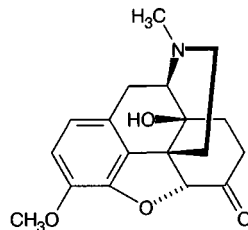
Wells, D.S.; Janssen, F.W.; Ruelius, H.W. Interactions between oxaprozin glucuronide and human serum albumin. *Xenobiotica*, **1987**, *17*, 1437–1449

Oxycodone

Molecular formula: C₁₈H₂₁NO₄

Molecular weight: 315.4

CAS Registry No.: 76-42-6, 124-90-3 (HCl), 64336-55-6 (terephthalate)



SAMPLE

Matrix: bile, blood

Sample preparation: 0.5 mL Blood or bile + 10 (blood) or 15 (bile) μ L 100 μ g/mL nalorphine in MeOH + 300 μ L 1.1 M pH 5.0 sodium acetate buffer + 3000-3500 U of *Patella vulgata* glucuronidase, incubate at 55° overnight, add 0.5 mL borate buffer to achieve a pH of 8.3-8.5. Add 8 mL chloroform:isopropanol 90:10, gently rotate for 30 min, centrifuge at 3500 rpm for 10 min, remove aqueous layer. Wash organic layer (twice for blood, three times for bile) with 3 mL 100 mM pH 9.9 sodium phosphate buffer with gentle rotation for 10 min and centrifugation each time. Add organic layer to 200 (blood) or 400 (bile) μ L 0.2% phosphoric acid, gently rotate for 30 min, discard organic layer, inject 50 μ L of the acid layer. (Borate buffer was 50 mM boric acid and 43 mM sodium tetraborate, adjusted to pH 9.8.)

HPLC VARIABLES

Guard column: Nova-Pak phenyl guard column

Column: 150 \times 3.9 5 μ m Nova-Pak phenyl

Mobile phase: MeCN:10 mM pH 6.6 NaH₂PO₄ 10:90

Flow rate: 1.2

Injection volume: 50

Detector: UV 210; F ex 220 em 370 (cut-off)

CHROMATOGRAM

Retention time: 21.4

Internal standard: nalorphine (23.5)

OTHER SUBSTANCES

Simultaneous: codeine, dihydrocodeine, hydrocodone, 6-monoacetylmorphine, morphine

Noninterfering: acetylcodeine, amitriptyline, amphetamine, diamorphine, diazepam, dothiepin, doxepin, ephedrine, ephedrine, hydromorphone, mesoridazine, methadone, methamphetamine, 3-monoacetylmorphine, nordiazepam, norpropoxyphene, nortriptyline, oxazepam, propoxyphene, pseudoephedrine, quinidine, quinine, sulfamethoxazole, sulforidazine, thioridazine

KEY WORDS

UV and F detection used together

REFERENCE

Crump, K.L.; McIntyre, I.M.; Drummer, O.H. Simultaneous determination of morphine and codeine in blood and bile using dual ultraviolet and fluorescence high-performance liquid chromatography. *J.Anal.Toxicol.*, **1994**, *18*, 208-212

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 60 μ L 1 μ g/mL codeine in water + 100 μ L 1 M phosphoric acid + 5 mL butyl chloride, mix for 1.5 min, centrifuge at 1500 g for 3 min, discard upper organic layer. To the aqueous layer add 500 μ L pH 10 1 M carbonate buffer

and 5 mL butyl chloride, mix for 1.5 min, centrifuge at 1500 g for 3 min, remove organic layer and repeat extraction. Combine butyl chloride layers and evaporate them to dryness under a stream of air at 40°. Reconstitute the residue in 200 μ L mobile phase, inject a 150 μ L aliquot.

HPLC VARIABLES

Guard column: Novapak C18 guard column

Column: 4 μ m Novapak C18 in a Waters RCM 8 \times 10 radial compression unit

Mobile phase: MeOH:MeCN:10 mM pH 7 phosphate buffer 230:20:1000, containing 40 mg/L cetyltrimethylammonium bromide (cetavlon)

Flow rate: 2

Injection volume: 150

Detector: E, Waters Model 460, working electrode 1.10 V

CHROMATOGRAM

Retention time: 8.5

Internal standard: codeine (13.5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, amitriptyline, aspirin, atenolol, camazepam, carbamazepine, chlorimipramine, chlorthalidone, clonazepam, cortisolone, desipramine, diazepam, halazepam, hydrochlorothiazide, hydrocortisone, imipramine, lorazepam, maprotiline, meperidine, methylphenobarbital, methylprednisolone, metoclopramide, midazolam, morphine, nalorphine, naloxone, nitrazepam, nortriptyline, oxazepam, oxprenolol, phenobarbital, phenytoin, pindolol, prazepam, prednisolone, prednisone, primidone, prochlorperazine, propranolol, salicylic acid, temazepam

KEY WORDS

plasma

REFERENCE

Smith, M.T.; Watt, J.A.; Mapp, G.P.; Cramond, T. Quantitation of oxycodone in human plasma using high-performance liquid chromatography with electrochemical detection. *Ther. Drug Monit.*, **1991**, *13*, 126-130

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 50 μ L 2 μ g/mL methadone in MeOH + 500 μ L pH 9.6 carbonate buffer + 6 mL butyl chloride, shake on a mechanical shaker at 100 rpm for 15 min, centrifuge at 2000 g for 5 min. Remove the organic layer and add it to 3 mL 200 mM HCl. Shake for 15 min, centrifuge, remove aqueous layer. Add aqueous layer to 3 drops 60% NaOH and 6 mL butyl chloride, shake for 15 min, centrifuge. Remove organic layer and evaporate it to dryness at 50° under a stream of nitrogen, reconstitute residue in 60 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 10 μ m Brownlee RP-8

Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 50:30:20

Flow rate: 1

Injection volume: 40

Detector: E, Bioanalytical Systems, glassy carbon working electrode 1.20 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6.4

Internal standard: methadone (16)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: fentanyl, meperidine, phenoperidine

KEY WORDS

plasma

REFERENCE

Schneider, J.J.; Triggs, E.J.; Bourne, D.W.; Stephens, I.D.; Haviland, A.M. Determination of oxycodone in human plasma by high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1984**, *308*, 359–362

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10 μ L aliquot of a 50 μ g/mL solution in water.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-ABZ

Mobile phase: MeCN:25 mM pH 7.5 potassium phosphate buffer 35:65

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Simultaneous: codeine, meperidine

REFERENCE

Supelco Chromatography Products, Supelco, Inc., Bellefonte PA, 1996, p. A23

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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), 5.82 (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, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, 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, sulfipyrazone, 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

some 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 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, dipyron, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 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: 2.670

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methypylon, N-norcodeine, oxazepam, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Asch, 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: 150 × 3.9 μBondapak C18

Mobile phase: MeCN:phosphate buffer 36:64, pH adjusted to 4.0

Flow rate: 1.5

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 2.5

Limit of detection: 50 ng/mL

REFERENCE

Tien, J.H. Transdermal-controlled administration of oxycodone. *J.Pharm.Sci.*, **1991**, 80, 741-743

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: 2.44 (tails)

OTHER SUBSTANCES

Simultaneous: benzphetamine, benzylmorphine, bromo-STP, buprenorphine, caffeine, codeine, codeine-N-oxide, dextromoramide, dextropropoxyphene, diamorphine, diethylpropion, dihydrocodeine, dihydromorphine, dimethylamphetamine, dipipanone, ephedrine, epinephrine, ethoheptazine, ethylmorphine, etorphine, fenethyline, fentanyl, hydrocodone, 4-hydroxyamphetamine, hydroxypethidine, levallorphan, levorphanol, mazindol, meperidine, mephentermine, mescaline, methadone, methamphetamine, methylephedrine, methylphenidate, morphine, morphine-3-glucuronide, morphine-N-oxide, nalorphine, naloxone, norcodeine, norlevorphanol, normetanephrine, normethadone, normorphine, norpethidine, norpipanone, noscapine, papaverine, pemoline, pentazocine, phenazocine, phendimetrazine, phenelzine, 2-phenethylamine, phenoperidine, phenylephrine, pholcodeine, pipradol, piritramide, prolintane, pseudoephedrine, STP, tranlycypromine, trimethoxyamphetamine, tyramine

Noninterfering: dopamine, levodopa, methyl dopa, methyl dopate, norepinephrine

Interfering: acetylcodeine, amphetamine, chlorphentermine, fencamfamin, fenfluramine, methylenedioxyamphetamine, monoacetylmorphine, norpseuoephedrine, phentermine, phenylpropanolamine, thebacon, thebaine

REFERENCE

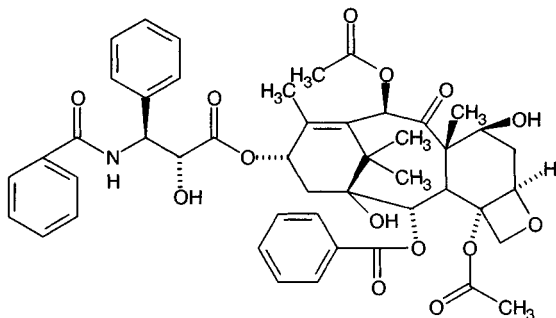
Law, B.; Gill, R.; Moffat, A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent. *J.Chromatogr.*, **1984**, *301*, 165-172

Paclitaxel

Molecular formula: C₄₇H₅₁NO₁₄

Molecular weight: 853.9

CAS Registry No.: 33069-62-4



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 300 ng N-nitrosodiphenylamine (Caution! N-Nitrosodiphenylamine is a carcinogen!) + 400 μ L 500 mM Na₂HPO₄, vortex for 5 s, add 5 mL ethyl acetate, mix for 1 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μ L mobile phase, vortex for 30 s, sonicate for 30 s, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: Guard-PAK C18 (Waters)

Column: 100 \times 8 4 μ m Nova Pak C18 radial compression

Mobile phase: MeCN:buffer 45.5:55.5 (Buffer was 1 mM Na₂HPO₄ adjusted to pH 5 with phosphoric acid.)

Flow rate: 4.5

Injection volume: 100

Detector: UV 227

CHROMATOGRAM

Retention time: 5.26

Internal standard: N-nitrosodiphenylamine (6.45)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: carmustine, 5-fluorouracil, hydrocortisone, teniposide, thiotepa

Noninterfering: acetaminophen, aspirin, bleomycin, busulfan, carboplatin, cyclophosphamide, cytarabine, dacarbazine, hydroxyurea, ifosfamide, methotrexate, mitomycin, mitoxantrone, procarbazine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

el-Yazigi, A.; Yusuf, A. Expedient liquid chromatographic assay for paclitaxel in plasma after its administration to cancer patients. *Ther. Drug Monit.*, **1995**, *17*, 511–515

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL cyano Bond Elut SPE cartridge with 2 mL MeOH and 2 mL 10 mM pH 5.0 ammonium acetate buffer. 1 mL Plasma + 1 mL 200 mM ammonium acetate, mix, add a 1 mL aliquot to the SPE cartridge, wash with 2 mL 10 mM pH 5.0 ammonium acetate, wash with 1 mL MeOH:10 mM pH 5.0 ammonium acetate 20:80, wash with 1 mL hexane, dry under vacuum for 1 min, elute with 2 mL MeCN:triethylamine 100:0.1, evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute with 200 μ L mobile phase, vortex for 30 s, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher RP-8

Column: 150 × 4.6 5 μm APEX-octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:20 mM pH 5.0 ammonium acetate buffer 40:10:50

Injection volume: 50

Detector: UV 227

CHROMATOGRAM

Retention time: 10.1

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; SPE

REFERENCE

Huizing, M.T.; Sparreboom, A.; Rosing, H.; van Tellingen, O.; Pinedo, H.M.; Beijnen, J.H. Quantification of paclitaxel metabolites in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *674*, 261–268

SAMPLE

Matrix: blood

Sample preparation: Plasma. 500 μL Plasma + 20 μL 10 μg/mL diethylstilbestrol in MeOH, vortex for 20 s, add 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. Microsomal incubations. 250 μL Microsomal incubation + 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. Wash is not necessary for microsomal incubations.)

Flow rate: 0.4

Injection volume: 50

Detector: UV 227

CHROMATOGRAM

Retention time: 22

Internal standard: diethylstilbestrol (17)

Limit of detection: 20 nM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

microsomal incubations; plasma; human; liver; pharmacokinetics

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.Pharm.Exp.Ther.*, **1995**, *275*, 566–575

SAMPLE

Matrix: blood

Sample preparation: Dilute 50–500 μL mouse plasma to 500 μL with water, add 25 μL 40 $\mu\text{g}/\text{mL}$ N-octylbenzamide in MeOH, mix, add 4 mL MTBE, vortex for 30 s, centrifuge at 500 g at 4° for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 70 μL aliquot. (Full details for the synthesis of N-octylbenzamide are given in the paper.)

HPLC VARIABLES

Guard column: 6.6 \times 3 10 μm $\mu\text{Bondapak C18}$

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeOH:water 70:30

Flow rate: 2

Injection volume: 70

Detector: UV 227

CHROMATOGRAM

Retention time: 7.35

Internal standard: N-octylbenzamide (13.00)

Limit of quantitation: 0.15 nM

KEY WORDS

plasma; mouse

REFERENCE

Sharma, A.; Conway, W.D.; Straubinger, R.M. Reversed-phase high-performance liquid chromatographic determination of taxol in mouse plasma. *J.Chromatogr.B*, **1994**, *655*, 315–319

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut cyano SPE cartridge with 2 mL MeOH and 2 mL 10 mM pH 5.0 ammonium acetate, do not allow to dry. 500 μL Plasma + 500 μL 200 mM pH 5.0 ammonium acetate, vortex for 20 s, add to the SPE cartridge, wash with 2 mL 10 mM pH 5.0 ammonium acetate, wash with 2 mL MeOH:10 mM pH 5.0 ammonium acetate 20:80, wash with 1 mL hexane, dry under vacuum for 1 min. Elute with two 1 mL volumes of 0.1% triethylamine in MeCN, evaporate the eluate to dryness at 30° under nitrogen, reconstitute the residue in 200 μL MeCN:MeOH:water 40:10:50 containing 10 mM ammonium acetate adjusted to pH 5.0 with glacial acetic acid, vortex for 30 s, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:water:1 M ammonium acetate adjusted to pH 5.0 with glacial acetic acid 40:10:49:1

Flow rate: 1

Injection volume: 100

Detector: UV 227

CHROMATOGRAM

Retention time: 10.6

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amsacrine, baccatin III, cephalomannine, daunorubicin, epipaclitaxel, paclitaxel C

KEY WORDS

plasma; SPE; pharmacokinetics; methyl paclitaxel can be used as IS

REFERENCE

Wiley, T.A.; Bekos, E.J.; Gaver, R.C.; Duncan, G.F.; Tay, L.K.; Beijnen, J.H.; Farmen, R.H. High-performance liquid chromatographic procedure for the quantitative determination of paclitaxel (Taxol) in human plasma. *J.Chromatogr.*, **1993**, *621*, 231-238

SAMPLE

Matrix: blood, culture medium, tissue

Sample preparation: Plasma, culture medium. 0.2-1 mL plasma or culture medium + 100 μ L 8.7 μ g/mL cephalomannine in MeOH + three volumes ethyl acetate, extract, repeat extraction. Combine extracts and centrifuge them at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100-150 μ L MeCN:water 37.5:62.5, inject a 10-60 μ L aliquot onto column A with mobile phase A and elute to waste. After 8 min direct the effluent from column A onto column B. After another 7 min elute column B with mobile phase B, monitor the effluent from column B, re-equilibrate column A with mobile phase A. Tissue. 40 mg Dog bladder tissue + 100 μ L 8.7 μ g/mL cephalomannine in MeOH + 3-4 mL ethyl acetate or MeCN, homogenize (Tekmar) for 1 min, wash homogenizer probe with 3-4 mL same solvent for 15-20 s. Combine organic layers and centrifuge them at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100-150 μ L MeCN:water 37.5:62.5, inject a 10-60 μ L aliquot onto column A with mobile phase A and elute to waste. After 8 min direct the effluent from column A onto column B. After another 7 min 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 Nova-Pak C8 guard column + 75 \times 3.9 3 μ m Nova-Pak C8; B 250 \times 4.6 5 μ m Bakerbond C18

Mobile phase: A MeCN:water 37.5:62.5; B MeCN:water 49:51

Flow rate: A 1; B 1.2

Injection volume: 10-60

Detector: UV 229

CHROMATOGRAM

Retention time: 21.35

Internal standard: cephalomannine (20.11)

Limit of detection: 5 ng/mL (plasma); 5 ng/inj. (tissue)

KEY WORDS

plasma; column-switching; dog; bladder; heart-cut

REFERENCE

Song, D.; Au, J.L.-S. Isocratic high-performance liquid chromatographic assay of taxol in biological fluids and tissues using automated column switching. *J.Chromatogr.B*, **1995**, *663*, 337-344

SAMPLE

Matrix: blood, feces, tissue, urine

Sample preparation: Condition a 1 mL 100 mg Bond Elut cyano SPE cartridge with 2 mL MeOH and 2 mL 10 mM pH 5.0 ammonium acetate buffer. Dilute urine with five volumes of human plasma. Homogenize (Biospec) tissue or feces in 5-10 volumes of 4% Boseral in

water at 4°. 200 (Feces, plasma), 200-1000 (tissue), or 1000 (urine) μL + 25 μL 20 $\mu\text{g}/\text{mL}$ 2'-methylpaclitaxel in MeOH + 4 mL diethyl ether, mix vigorously for 5 min, centrifuge at 460 g for 5 min, freeze in EtOH/dry ice, repeat the extraction. Combine the organic layers and evaporate them to dryness under vacuum at 43°, reconstitute in 250 μL human plasma, add 300 μL 200 mM pH 5.0 ammonium acetate buffer, mix, add 500 μL to the SPE cartridge, wash with 2 mL 10 mM pH 5.0 ammonium acetate buffer, wash with 1 mL MeOH:10 mM pH 5.0 ammonium acetate buffer 20:80, elute with 500 μL MeCN:triethylamine 100:0.1. Evaporate the eluate to dryness under vacuum, reconstitute 200 μL in MeCN:MeOH:water 40:10:50, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μm APEX-octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:200 mM pH 5.0 ammonium acetate buffer 40:10:50

Flow rate: 1

Injection volume: 50

Detector: UV 227

CHROMATOGRAM

Retention time: 10.6

Internal standard: 2'-methylpaclitaxel (15.3)

Limit of detection: 15 ng/mL (plasma)

Limit of quantitation: 25-125 ng/g

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; mouse; SPE; brain; fat; muscle; breast; colon; appendix; intestine; stomach; liver; gall bladder; kidney; lung; spleen; heart; uterus; ovary; thymus; lymph nodes

REFERENCE

Sparreboom, A.; van Tellingen, O.; Nootjen, W.J.; Beijnen, J.H. Determination of paclitaxel and metabolites in mouse plasma, tissues, urine and faeces by semi-automated reversed-phase high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *664*, 383-391

SAMPLE

Matrix: blood, urine

Sample preparation: 800 μL Plasma or urine + 200 μL 7.5 μM N-cyclohexylbenzamide in MeCN, vortex, add 3 mL ethyl acetate, shake vigorously for 2 min, centrifuge at 150 g for 2 min. Remove the organic layer and evaporate it under nitrogen at room temperature, dissolve the residue in 100 μL MeCN, inject an aliquot.

HPLC VARIABLES

Guard column: C-18 Guard-Pak

Column: 100 \times 8 10 μm C18 Radial-Pak

Mobile phase: MeCN:water from 35:65 to 100:0 exponentially over 20 min (Waters exponential gradient curve no. 9), keep at 100:0 for 7 min, re-equilibrate at 35:65 for 5 min.

Flow rate: 2.5

Detector: UV 227

CHROMATOGRAM

Retention time: 19.5

Internal standard: N-cyclohexylbenzamide (12.5)

Limit of quantitation: 30 nM

KEY WORDS

plasma

REFERENCE

Longnecker, S.M.; Donehower, R.C.; Cates, A.E.; Chen, T.-L.; Brundrett, R.B.; Grochow, L.B.; Ettinger, D.S.; Colvin, M. High-performance liquid chromatographic assay for taxol in human plasma and urine and pharmacokinetics in a phase I trial. *Cancer Treat.Rep.*, **1987**, *71*, 53–59

SAMPLE

Matrix: cell cultures

Sample preparation: Filter (Miracloth, Calbiochem). Evaporate 1 mL of filtrate to dryness under vacuum, reconstitute in 200 μ L MeOH:acetic acid 99.99:0.01, sonicate for 1 min, centrifuge at 16000 g for 15 min, filter (0.2 μ m PVDF), inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: Upchurch ODS

Column: 250 \times 4.6 4 μ m Curosil G (Phenomenex)

Mobile phase: MeCN:water 52.5:47.5

Flow rate: 1

Detector: UV 228

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Simultaneous: impurities, related compounds

REFERENCE

Ketchum, R.E.B.; Gibson, D.M. A novel method of isolating taxanes from cell suspension cultures of yew (*Taxus* spp.). *J.Liq.Chromatogr.*, **1995**, *18*, 1093–1111

SAMPLE

Matrix: cell cultures

Sample preparation: Homogenize (Omni-mix) 50 mg bark or foliage in 1.5 mL MeOH for 2 min, sonicate for 5 min, centrifuge, filter (0.2 μ m) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: phenyl (Rainin)

Column: 250 \times 4.6 8 μ m Dynamax 60 Å phenyl (Rainin)

Mobile phase: MeOH:MeCN:50 mM pH 4.4 acetate buffer 20:39:41

Flow rate: 1

Detector: UV 227

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Extracted: cephalomannine

REFERENCE

Wickremesinha, E.R.M.; Arteca, R.N. Methodology for the identification and purification of taxol and cephalomannine from *Taxus callus* cultures. *J.Liq.Chromatogr.*, **1993**, *16*, 3263–3274

SAMPLE

Matrix: formulations

Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18
Mobile phase: MeCN:water 40:60
Flow rate: 2.25
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.95

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 1:4, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:12.5 mM ammonium phosphate 60:40, pH adjusted to 4.5 with 1 M HCl
Flow rate: 1
Injection volume: 20
Detector: UV 227

CHROMATOGRAM

Retention time: 10.0

OTHER SUBSTANCES

Noninterfering: ondansetron, ranitidine

KEY WORDS

injections; 5% dextrose; stability-indicating

REFERENCE

Burm, J.-P.; Jhee, S.S.; Chin, A.; Moon, Y.S.K.; Jeong, E.; Nii, L.; Fox, J.L.; Gill, M.A. Stability of paclitaxel with ondansetron hydrochloride or ranitidine hydrochloride during simulated Y-site administration. *Am.J.Hosp.Pharm.*, 1994, 51, 1201–1204

SAMPLE

Matrix: formulations
Sample preparation: Filter (5 μm), dilute 1 mg/mL solutions tenfold with the same solvent, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac C18
Mobile phase: MeCN:water 53:47
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM**Retention time:** 6.09

KEY WORDSinjections; 5% dextrose; saline; stability-indicating

REFERENCEXu, Q.; Trissel, L.A.; Martinez, J.F. Stability of paclitaxel in 5% dextrose injection or 0.9% sodium chloride injection at 4, 22, or 32°C. *Am.J.Hosp.Pharm.*, **1994**, *51*, 3058–3060

SAMPLE**Matrix:** microsomal incubations**Sample preparation:** Extract microsomal incubation with 5 volumes ethyl acetate, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue in 500 µL mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 3.2 6 µm Curosil G (Phenomenex)**Mobile phase:** MeCN:water 35:65**Flow rate:** 0.6**Detector:** UV 229

CHROMATOGRAM**Retention time:** 58

OTHER SUBSTANCES**Extracted:** metabolites

KEY WORDSrat; liver

REFERENCEAnderson, C.D.; Wang, J.; Kumar, G.N.; McMillan, J.M.; Walle, U.K.; Walle, T. Dexamethasone induction of taxol metabolism in the rat. *Drug Metab.Dispos.*, **1995**, *23*, 1286–1290

SAMPLE**Matrix:** plants**Sample preparation:** Extract needles with MeOH, concentrate the extract under reduced pressure at <30°, partition concentrate with 0.8 volumes of water and 0.8 volumes of chloroform, repeat the extraction with 0.6 volumes chloroform then 0.4 volumes chloroform, concentrate under reduced pressure, dry under vacuum at 35–40°, dissolve in MeCN/water, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 µm Partisil C8**Mobile phase:** MeCN:MeOH:water 50:10:40**Flow rate:** 0.5**Detector:** UV 254

KEY WORDSneedles; details of preparative HPLC also in paper

REFERENCERao, K.V.; Bhakuni, R.S.; Juchum, J.; Davies, R.M. A large scale process for paclitaxel and other taxanes from the needles of *Taxus x media Hicksii* and *Taxus floridana* using reverse phase column chromatography. *J.Liq.Chrom.Rel.Technol.*, **1996**, *19*, 427–447

SAMPLE**Matrix:** plants**Sample preparation:** Shake 2 g ground plant material and 100 mL MeOH on a flat-bed orbital shaker for 12-16 h, allow to settle. Remove a 10 mL aliquot and add it to 10 mL 5% NaCl solution, wash twice with 10 mL portions of hexane, discard the hexane washes, extract four times with 10 mL portions of dichloromethane. Filter the dichloromethane extracts through 3 g anhydrous sodium sulfate, evaporate the filtrate to dryness, reconstitute with 2 mL dichloromethane, add to a 250 × 10 column dry packed with 1 g 149-250 µm Davsil silica (Alltech), wash with two 2 mL portions of dichloromethane, wash with two 5 mL portions of acetone:dichloromethane 4:96, elute with two 5 mL portions of acetone:dichloromethane 50:50. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 1 mL MeOH, inject a 10 µL aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 5 µm Zorbax SB-C8**Mobile phase:** Gradient. A was MeCN. B was MeOH:water 20:80. A:B 20:80 for 5 min, to 25:75 over 0.1 min, maintain at 25:75 for 8.9 min, to 35:65 over 10 min, maintain at 35:65 for 17 min, to 65:35 over 3 min, maintain at 65:35 for 11 min, return to initial conditions over 5 min, re-equilibrate for 20 min.**Column temperature:** 35**Flow rate:** 1**Injection volume:** 10**Detector:** UV 227

CHROMATOGRAM**Retention time:** 42.5**Limit of detection:** 660 ng/g

OTHER SUBSTANCES**Extracted:** baccatin III, cephalomannine

KEY WORDS

SPE; leaves; twigs

REFERENCELauren, D.R.; Jensen, D.J.; Douglas, J.A. Analysis of taxol, 10-deacetylbaaccatin III and related compounds in *Taxus baccata*. *J.Chromatogr.A*, **1995**, 712, 303-309

SAMPLE**Matrix:** plants**Sample preparation:** Homogenize (Omni-mix) 50 mg bark or foliage in 1.5 mL MeOH for 2 min, sonicate for 5 min, centrifuge, filter (0.2 µm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES**Column:** 1000 × 4.7 7 µm porous graphitized carbon (Shandon)**Mobile phase:** Dioxane: water 46:54 (Caution! Dioxane is a carcinogen!)**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 228

CHROMATOGRAM**Retention time:** 18**Limit of detection:** 30 ng/mL

KEY WORDS

bark; foliage

REFERENCE

Forgács, E. Use of porous graphitized carbon column for determining taxol in *Taxus baccata*. *Chromatographia*, **1994**, *39*, 740–742

SAMPLE**Matrix:** plants

Sample preparation: Grind yew needles to <3 mm in a blender. Weigh out 3–4 g, add 100 mL MeOH, shake for 16 h on a wrist-action shaker, filter (Whatman 1 or 2 paper), wash the solid with 25 mL MeOH. Evaporate the filtrate to dryness under reduced pressure at 40–43°, reconstitute the residue in 10 mL MeOH and 1 mL water. Condition a 47 mm Empore SPE extraction disk (3M Corp.) with 15 mL ethyl acetate, 15 mL MeOH and 15 mL water. Use a 47 mm polypropylene separator with 10 µm pore size (Gelman 61757) in front of the extraction disk. Pass 10 mL water and 7 mL crude extract through the disk, wash with 15 mL water, wash with 15 mL MeOH:water 20:80, 15 mL MeOH:water 45:55, elute with 20 mL MeOH:water 80:20, filter (2 µm) the eluate, inject a 10 µL aliquot.

HPLC VARIABLES**Guard column:** Taxsil guard cartridge (0335-CS) (MetaChem)**Column:** 250 × 4.6 5 µm Taxsil (0335-250 × 046) (MetaChem)**Mobile phase:** Gradient. A was MeCN. B was MeOH:water 30:70. A:B 41:59 for 15 min, to 65:35 over 5 min, maintain at 65:35 for 10 min, to 41:59 over 5 min, maintain at 41:59 for 5 min.**Column temperature:** 32**Flow rate:** 1**Injection volume:** 10**Detector:** UV 230

CHROMATOGRAM**Retention time:** 10

KEY WORDS

SPE

REFERENCE

Mattina, M.J.I.; MacEachern, G.J. Extraction, purification by solid-phase extraction and high-performance liquid chromatographic analysis of taxanes from ornamental *Taxus* needles. *J.Chromatogr.A*, **1994**, *679*, 269–275

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 5 µm LiChrospher diol**Mobile phase:** Gradient. MeOH:carbon dioxide 8:92 for 3 min, to 28:72 over 25 min, to 35:65 over 5.7 min, maintain at 35:65 for 4 min.**Column temperature:** 30**Flow rate:** 2**Detector:** UV 227

CHROMATOGRAM**Retention time:** 15.77

OTHER SUBSTANCES

Simultaneous: impurities, degradation products

KEY WORDS

SFC; pressure 150 bar

REFERENCE

Jagota, N.K.; Nair, J.B.; Frazer, R.; Klee, M.; Wang, M.Z. Supercritical fluid chromatography of paclitaxel. *J.Chromatogr.A*, **1996**, *721*, 315–322

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: C-18 Guard Pak

Column: 100 \times 8 10 μ m Resolve C-18 radial compression (Waters)

Mobile phase: Gradient. MeCN:water from 45:55 to 100:0 over 20 min (exponential gradient), maintain at 100:0 for 5 min, re-equilibrate at initial conditions for 5 min.

Column temperature: 21

Flow rate: 2.5

Injection volume: 20

Detector: UV 227

CHROMATOGRAM

Retention time: 9.33

REFERENCE

Wenk, M.R.; Fahr, A.; Reszka, R.; Seelig, J. Paclitaxel partitioning into lipid bilayers. *J.Pharm.Sci.*, **1996**, *85*, 228–231

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Partisil C8

Mobile phase: MeCN:MeOH:water 50:10:40

Flow rate: 0.5

Detector: UV 254

CHROMATOGRAM

Retention time: 17

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

details of preparative chromatography

REFERENCE

Rao, K.V.; Hanuman, J.B.; Alvarez, C.; Stoy, M.; Juchum, J.; Davies, R.M.; Baxley, R. A new large-scale process for taxol and related taxanes from *Taxus brevifolia*. *Pharm.Res.*, **1995**, *12*, 1003–1010

SAMPLE

Matrix: urine

Sample preparation: 19 mL Urine + 1 mL Cremophor EL:EtOH 50:50 (Sigma), mix. 500 μ L Sample + 500 μ L 200 mM pH 5.0 ammonium acetate buffer + 5 mL n-butyl chloride, rotate slowly for 10 min, centrifuge at 2500 g for 10 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L MeCN:MeOH:water 40:50:10 (40:10:50 ?), vortex for 30 s, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m LiChrospher RP-8

Column: 150 \times 4.6 5 μ m APEX-octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:20 mM pH 5.0 ammonium acetate buffer 40:10:50

Flow rate: 1

Injection volume: 50

Detector: UV 227

CHROMATOGRAM

Retention time: 10

Limit of quantitation: 250 ng/mL

REFERENCE

Huizing, M.T.; Rosing, H.; Koopman, F.; Keung, A.C.F.; Pinedo, H.M.; Beijnen, J.H. High-performance liquid chromatographic procedures for the quantitative determination of paclitaxel (Taxol) in human urine. *J.Chromatogr.B*, **1995**, *664*, 373–382

SAMPLE

Matrix: urine

Sample preparation: Condition a 1 mL 100 mg Bond Elut cyano SPE cartridge with 2 mL MeOH and 2 mL 10 mM pH 5.0 ammonium acetate buffer. 19 mL Urine + 1 mL Cremophor EL:EtOH 50:50 (Sigma), mix. 500 μ L Sample + 500 μ L 200 mM pH 5.0 ammonium acetate buffer, vortex for 20 s, add to the SPE cartridge, wash with 2 mL 10 mM pH 5.0 ammonium acetate buffer, wash with 1 mL MeOH:10 mM pH 5.0 ammonium acetate buffer 20:80, wash with 1 mL hexane, dry under vacuum for 1 min, elute with 2 mL MeCN:triethylamine 100:0.1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute in 200 μ L MeCN:MeOH:water 40:50:10 (40:10:50 ?), vortex for 30 s, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m LiChrospher RP-8

Column: 150 \times 4.6 5 μ m APEX-octyl (Jones Chromatography)

Mobile phase: MeCN:MeOH:20 mM pH 5.0 ammonium acetate buffer 40:10:50

Flow rate: 1

Injection volume: 50

Detector: UV 227

CHROMATOGRAM

Retention time: 10

Limit of detection: 8 ng/mL

Limit of quantitation: 10 ng/mL

KEY WORDS

SPE

REFERENCE

Huizing, M.T.; Rosing, H.; Koopman, F.; Keung, A.C.F.; Pinedo, H.M.; Beijnen, J.H. High-performance liquid chromatographic procedures for the quantitative determination of paclitaxel (Taxol) in human urine. *J.Chromatogr.B*, **1995**, *664*, 373–382

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- Alkan-Onyuksel, H.; Ramakrishnan, S.; Chai, H.-B.; Pezzuto, J.M. Mixed micellar formulation suitable for the parenteral administration of taxol. *Pharm.Res.*, **1994**, *11*, 206–212 [formulations]
- Klecker, R.W.; Jamis-Dow, C.A.; Egorin, M.J.; Erkmen, K.; Parker, R.J.; Stevens, R.; Collins, J.M. Effect of cimetidine, probenecid, and ketoconazole on the distribution, biliary secretion, and metabolism of [³H]taxol in the Sprague-Dawley rat. *Drug Metab.Dispos.*, **1994**, *22*, 254–258 [rat; plasma; bile; urine; lung; spleen; liver; kidney; heart; muscle; brain; testes; fat; UV detection; radioactivity detection; tritium labeled]
- Kopycki, W.J.; ElSohly, H.N.; McChesney, J.D. HPLC determination of taxol and related compounds in *Taxus* plant extracts. *J.Liq.Chromatogr.*, **1994**, *17*, 2569–2591 [gradient]
- Kumar, G.N.; Walle, U.K.; Walle, T. Cytochrome P450 3A-mediated human liver microsomal taxol 6 α -hydroxylation. *J.Pharmacol.Exp.Ther.*, **1994**, *268*, 1160–1165 [microsomal incubations; extracted metabolites]
- Mase, H.; Hiraoka, M.; Suzuki, A.; Nakanomyo, H. [Determination of new anticancer drug, paclitaxel, in biological fluids by high performance liquid chromatography]. *Yakugaku.Zasshi.*, **1994**, *114*, 351–355
- Bitsch, F.; Ma, W.; Macdonald, F.; Nieder, M.; Shackleton, C.H. Analysis of taxol and related diterpenoids from cell cultures by liquid chromatography-electrospray mass spectrometry. *J.Chromatogr.*, **1993**, *615*, 273–280 [LC-MS; electrospray; microbore; gradient; LOD 100 pg]
- Castor, T.P.; Tyler, T.A. Determination of taxol in *Taxus media* needles in the presence of interfering components. *J.Liq.Chromatogr.*, **1993**, *16*, 723–731 [SPE; plants; simultaneous baccatin, cephalomannine]
- Ketchum, E.B.; Gibson, D.M. Rapid isocratic reversed phase HPLC of taxanes on new columns developed specifically for taxol analysis. *J.Liq.Chromatogr.*, **1993**, *16*, 2519–2530 [simultaneous baccatin, cephalomannine; gradient; column temp 30]
- Wheeler, N.C.; Jech, K.; Masters, S.; Brobst, S.W.; Alvarado, A.B.; Hoover, A.J.; Snader, K.M. Effects of genetic, epigenetic, and environmental factors on taxol content in *Taxus brevifolia* and related species. *J.Nat.Prod.*, **1992**, *55*, 432–440 [plants]
- Waugh, W.N.; Trissel, L.A.; Stella, V.J. Stability, compatibility, and plasticizer extraction of taxol (NSC-125973) injection diluted in infusion solutions and stored in various containers. *Am.J.Hosp.Pharm.*, **1991**, *48*, 1520–1524 [stability-indicating; simultaneous degradation products; injections; 5% dextrose; saline]
- Monsarrat, B.; Mariel, E.; Cros, S.; Garès, M.; Guénard, D.; Guëritte-Voegelein, F.; Wright, M. Taxol metabolism. Isolation and identification of three major metabolites of taxol in rat bile. *Drug Metab.Dispos.*, **1990**, *18*, 895–901 [rat; bile; extracted metabolites]
- Witherup, K.M.; Look, S.A.; Stasko, M.W.; Ghiorzi, T.J.; Muschik, G.M.; Cragg, G.M. *Taxus* spp. needles contain amounts of taxol comparable to the bark of *Taxus brevifolia*: analysis and isolation. *J.Nat.Prod.*, **1990**, *53*, 1249–1255 [plants; analytical; preparative]
- Witherup, K.M.; Look, S.A.; Stasko, M.W.; McCloud, T.G.; Issaq, H.J.; Muschik, G.M. High performance liquid chromatographic separation of taxol and related compounds from *Taxus brevifolia*. *J.Liq.Chromatogr.*, **1989**, *12*, 2117–2132 [plants; simultaneous baccatin, cephalomannine; gradient; isocratic]
- Xu, L.X.; Liu, A.R. Determination of taxol in the extract of *taxus chinensis* by reversed phase HPLC. *Yao Hsueh Hsueh Pao*, **1989**, *24*, 552–555

Penicillin V

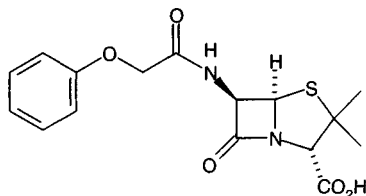
Molecular formula: C₁₆H₁₈N₂O₅S

Molecular weight: 350.4

CAS Registry No.: 87-08-1, 132-98-9 (potassium salt),

5928-84-7 (benzathine), 63690-57-3 (benzathine

tetrahydrate), 6591-72-6 (hydrabamine)



SAMPLE

Matrix: blood

Sample preparation: Condition a Baker C18 SPE cartridge with 5 mL water and 5 mL 2% NaCl, do not allow to run dry. 2 mL Plasma + 30 mL water + 2 mL 170 mM sulfuric acid + 2 mL 5% sodium tungstate solution, vortex for 30 s, centrifuge at 2200 g for 10 min, filter supernatant (GF/B glass fiber filter), add 10 mL 20% NaCl, mix, add to the SPE cartridge at 3 mL/min, wash with 5 mL 2% NaCl, wash with 5 mL water, draw air through cartridge for 5 min, elute with 500 μ L elution solution. Add 500 μ L derivatization reagent to the eluate, vortex for 20 s, allow to react at 65° for 30 min, cool to room temperature, vortex, filter (0.45 μ m), inject 50-100 μ L aliquots. (Prepare derivatization reagent by dissolving 34.45 g 1,2,4-triazole in 150 mL water, add 25 mL 10 mM mercuric chloride solution, mix, adjust the pH to 9.0 \pm 0.5 with 5 M NaOH, dilute to 250 mL with water. Prepare elution solution by mixing 60 mL MeCN and 5 mL buffer and making up to 100 mL with water. The buffer was 0.994 g Na₂HPO₄ + 1.794 g NaH₂PO₄·H₂O in 100 mL water, pH 6.5.)

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Nova-Pak C18

Mobile phase: MeCN:buffer 25:75 (Buffer contained 4.969 g Na₂HPO₄ + 8.969 g NaH₂PO₄·H₂O + 2.482 g anhydrous sodium thiosulfate per liter.)

Flow rate: 1

Injection volume: 50-100

Detector: UV 325

CHROMATOGRAM

Retention time: 5.8

Internal standard: penicillin V

OTHER SUBSTANCES

Extracted: penicillin G

KEY WORDS

plasma; cow; SPE; penicillin V is IS

REFERENCE

Boison, J.O.; Korsrud, G.O.; MacNeil, J.D.; Keng, L.; Papich, M. Determination of penicillin G in bovine plasma by high-performance liquid chromatography after pre-column derivatization. *J. Chromatogr.*, 1992, 576, 315-320

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 100 μ L 50 μ g/mL chloramphenicol in water + 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 μ L water, inject a 50 μ L 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: 3.10

Internal standard: chloramphenicol (5.10)

Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Noninterfering: amikacin, amiloride, amoxicillin, ampicillin, cephalexin, doxycycline, ethosuximide, gentamicin, hydrochlorothiazide, netilmicin, phenacetin, phenemal, phenytoin, primidone, sisomicin, tetracycline, tobramycin

Interfering: cloxacillin, penicillin G procaine

KEY WORDS

serum

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, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μL 100 μg/mL oxacillin in water + 20 μL 4% aqueous sodium dodecyl hydrogen sulfate solution, shake for 30 min, filter (Amicon MPS-1 micropartition system, YMT membrane) while centrifuging, adjust the pH of the ultrafiltrate to 6.3-6.5 with pH 4 citrate buffer, inject a 500 μL aliquot onto column A with mobile phase A and elute to waste, after 10 min elute the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. Urine. 5-100 μL Urine + 50 μL 100 μg/mL oxacillin in water, make up to 500 μL with water, inject onto column A with mobile phase A and elute to waste, after 10 min elute 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 50 × 4 Nucleosil 5-C18; B 250 × 5 Nucleosil 5-C18

Mobile phase: A MeCN:33 mM NaH₂PO₄ 5:95; B MeCN:33 mM NaH₂PO₄ 20:80

Injection volume: 500

Detector: UV 210

CHROMATOGRAM

Internal standard: oxacillin

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; column-switching; pharmacokinetics; ultrafiltrate

REFERENCE

Lintz, W.; Hirsch, I.; Osterloh, G.; Schmidt-Böthelt, E.; Sous, H. Bioverfügbarkeit von Penicillin V in einer wässrigen Zubereitungsform [Bioavailability of penicillin V in aqueous dosage forms]. *Arzneimittelforschung*, **1984**, *34*, 66–71

SAMPLE

Matrix: cheese, milk, yogurt

Sample preparation: Condition a 6 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 (or 5 g yogurt or cottage cheese + 4 mL 1 M pH 6 phosphate buffer) + 25 mL water + 4 mL 170 mM sulfuric acid + 40 mL 5% sodium tungstate, vortex for 30 s, centrifuge at 1500 g for 10 min, remove the supernatant, add 10 mL 20% NaCl to the residue, vortex for 10 s, centrifuge. Combine the supernatants and add them to the SPE cartridge, wash with 10 mL 2% NaCl, wash with 10 mL water, elute with 1 mL MeCN:200 mM pH 6.5 sodium phosphate buffer:water 60:5:35. Add 1 mL reagent to the eluate, vortex for 10 s, heat at 65° for 30 min, cool to room temperature, vortex, filter (Acro 0.45 μ m), inject a 50-100 μ L aliquot of the filtrate. (Prepare reagent by dissolving 34.45 g 1,2,4-triazole in 150 mL water, add 25 mL 10 mM mercuric chloride solution, mix, adjust pH to 9.0 \pm 0.5 with 5 M NaOH, make up to 250 mL with water.)

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Nova-Pak C18

Mobile phase: MeCN:buffer 25:75 (Buffer was 4.696 g Na₂HPO₄, 8.969 g NaH₂PO₄·H₂O, and 2.482 g anhydrous sodium thiosulfate in 1 L water.)

Flow rate: 0.8

Injection volume: 50-100

Detector: UV 325

CHROMATOGRAM

Retention time: 7

Internal standard: penicillin V

OTHER SUBSTANCES

Extracted: penicillin G

KEY WORDS

derivatization; cow; SPE; penicillin V is IS

REFERENCE

Boison, J.O.K.; Keng, L.J.-Y.; MacNeil, J.D. Analysis of penicillin G in milk by liquid chromatography. *JAOAC Int.*, **1994**, *77*, 565-570

SAMPLE

Matrix: cheese, milk, yogurt

Sample preparation: Condition a 6 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 (or 5 g yogurt or cottage cheese + 4 mL 1 M pH 6 phosphate buffer) + 25 mL water + 4 mL 170 mM sulfuric acid + 40 mL 5% sodium tungstate, vortex for 30 s, centrifuge at 1500 g for 10 min, remove the supernatant, add 10 mL 20% NaCl to the residue, vortex for 10 s, centrifuge. Combine the supernatants and add them to the SPE cartridge, wash with 10 mL 2% NaCl, wash with 10 mL water, elute with 750 μ L MeCN:200 mM ammonium acetate:water 60:5:35, filter (Acro 0.45 μ m), inject a 50-100 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Nova-Pak C18

Mobile phase: Gradient. A was MeCN. B was MeCN:150 mM ammonium acetate 10:90. A:B 0:100 for 10 min, to 30:70 over 10 min, return to initial conditions over 10 min.

Flow rate: 0.9

Injection volume: 50-100

Detector: MS, VG Trio II, probe tip 255°, source 180°, thermospray/plasmaspray, m/z 335, m/z 160

CHROMATOGRAM**Internal standard:** penicillin V

OTHER SUBSTANCES**Extracted:** penicillin G

KEY WORDS

cow; SPE; penicillin V is IS

REFERENCE

Boison, J.O.K.; Keng, L.J.-Y.; MacNeil, J.D. Analysis of penicillin G in milk by liquid chromatography. *J.AOAC Int.*, **1994**, *77*, 565–570

SAMPLE**Matrix:** fermentation broth

Sample preparation: Adjust pH of fermentation broth to 7, centrifuge at 8000 g for 10 min, add MeCN, centrifuge, add dichloromethane to the supernatant, vortex for 10 s, shake for 15 min, centrifuge at 8000 g for 15 min. Add 1 mL of the aqueous layer to 100 μ L reagent, heat at 50° for 50 min, cool in an ice bath, inject a 20 μ L aliquot. (Prepare reagent by dissolving 4.125 g imidazole in 2.5 mL water, add 1 mL HCl, add 500 μ L 110 mM mercury(II) chloride, add 1.5 mL HCl. Recrystallize imidazole twice from isopropanol.)

HPLC VARIABLES**Guard column:** 10 \times 4.5 μ m Spherisorb C18**Column:** 20 \times 4.6 μ m Spherisorb C18 S5ODS2

Mobile phase: Gradient. MeCN:buffer from 16.5:83.5 to 31.5:68.5 over 17 min (Buffer was 10 mM NaH₂PO₄ containing 10 mM EDTA, adjusted to pH 6.5 with 2 M NaOH.)

Flow rate: 2**Injection volume:** 20**Detector:** UV 325

CHROMATOGRAM**Retention time:** 14.5**Limit of detection:** 1 μ g/mL

OTHER SUBSTANCES**Extracted:** methicillin, penicillin G, penicillin X

KEY WORDS

derivatization

REFERENCE

Rogers, M.E.; Adlard, M.W.; Saunders, G.; Holt, G. High-performance liquid chromatographic determination of penicillins following derivatization to mercury-stabilized penicillenic acids. *J.Liq. Chromatogr.*, **1983**, *6*, 2019–2031

SAMPLE**Matrix:** formulations

Sample preparation: Powder 20 tablets, dissolve a portion of the powder in water such that the concentration of penicillin V potassium is 0.6 mg/mL, mix well, filter. Mix 20 mL filtrate with 15 mL 0.4 mg/mL sulfadimethoxine in MeCN:water 50:50, dilute to 100 mL with water, mix well, inject a 20 μ L aliquot.

HPLC VARIABLES**Guard column:** 40 mm long 30-50 μ m Whatman Co:Pell ODS

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:10 mM KH₂PO₄ 21:4:75
Flow rate: 1-1.5
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Internal standard: sulfadimethoxine

KEY WORDS

tablets; collaborative study

REFERENCE

Mopper, B. Liquid chromatographic determination of penicillin V potassium in tablets: collaborative study. *J.Assoc.Off.Anal.Chem.*, **1989**, 72, 883-884

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 KH₂PO₄ 19:11:70

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 8.4

Limit of detection: 1.02 μg/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G

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: 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 KH₂PO₄ 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 penicillin V (25 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 33:67 (Buffer was 5 mM phosphoric acid and 5 mM KH₂PO₄.)

Flow rate: 1

Injection volume: 200

Detector: UV 210

CHROMATOGRAM

Limit of quantitation: 2-5 ppb; cow

OTHER SUBSTANCES

Extracted: amoxicillin, ampicillin, ceftiofur, cephalirin, cloxacillin, penicillin G

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: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of dichloromethane:hexane 50:50, 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 10μm 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, thin layer cell, 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: 11.8

Limit of detection: 0.2 ppm

OTHER SUBSTANCES

Extracted: ampicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G

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: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 mL 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 centri-

fuging 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% NaHCO₃ 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% NaHCO₃ 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: 6.73

Internal standard: benzaldehyde (derivatized) (12.18)

Limit of detection: 5 ng/g

OTHER SUBSTANCES

Extracted: cloxacillin, dicloxacillin, methicillin, nafcillin, penicillin G, phenethicillin

Interfering: oxacillin

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: Condition a Sep-Pak C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 2 mL 2% NaCl. Pass through 30 g filtered (glass-wool plug) milk at 2 mL/min, wash with 5 mL water, wash with 10 mL MeOH:water:20% NaCl 10:80:10 containing 20 mM 18-crown-6, elute with 10 mL 15% (v/v) MeOH, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 50 × 2.1 Permaphase ETH (Du Pont)

Column: 150 × 4.3 LiChrosorb RP-18

Mobile phase: MeOH:water:0.2 M pH 4.0 phosphate buffer 25:65:10 containing 11 mM sodium 1-heptanesulfonate

Column temperature: 45

Flow rate: 1

Injection volume: 100

Detector: UV 210

CHROMATOGRAM

Retention time: 18.5

Limit of detection: 30 ng/g

OTHER SUBSTANCES

Extracted: ampicillin, penicillin G

KEY WORDS

cow; SPE

REFERENCE

Terada, H.; Sakabe, Y. Studies on residual antibacterials in foods. IV. Simultaneous determination of penicillin G, penicillin V and ampicillin in milk by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, *348*, 379-387

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 25:75

Flow rate: 1.5

Injection volume: 10-20

Detector: UV 220

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: 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: carbenicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, penicillin G

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: tissue

Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 7.2 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 phases, centrifuge, inject a 200 μ L aliquot onto 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 onto 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 \times 4 5 μ m LiChrospher 100 RP-18e; B 250 \times 4 5 μ m 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 \times 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 6.1

Limit of detection: 1.4 ng

OTHER SUBSTANCES

Extracted: cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS

post-column reaction; 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

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 μ L aliquot.

HPLC VARIABLES

Guard column: Polymer Labs guard cartridge

Column: 150 \times 4.6 5 μ m 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Labs)

Mobile phase: MeCN:buffer 18:82, after run was over flush at 35:65 for 5 min then re-equilibrate with 18:82 for 9 min. (Buffer was 10 mM pH 7 phosphate buffer prepared from 1.36 KH₂PO₄ and 2.84 g Na₂HPO₄ 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 10 mL 2% NaCl, do not allow to go dry. 5 g Tissue + 20 mL water, homogenize (Polytron, 20 mm probe), rinse probe with water so that total volume is 35 mL, shake mechanically for 5 min, add 5 mL 170 mM sulfuric acid, add 5 mL 5% sodium tungstate, vortex for 20 s, centrifuge at 2200 g for 10 min, remove the supernatant, add 15 mL water to the residue, shake for 5 min, centrifuge at 2200 g for 10 min. Combine the supernatants and filter (Whatman GF/B) them, add 10 mL 20% NaCl to the filtrate, mix thoroughly, add to the SPE cartridge at 3 mL/min, wash with 10 mL 2% NaCl, wash with 10 mL water, draw air through the cartridge for 5 min, immediately elute with 1 mL MeCN:200 mM pH 6.5 sodium phosphate buffer:water 60:5:35. Add 1 mL reagent to the eluate, vortex, heat at 65° for 30 min, cool rapidly to room temperature, vortex, filter (Acro 0.45 μ m), inject a 80-100 μ L aliquot of the filtrate. (Prepare reagent by dissolving 34.45 g 1,2,4-triazole in 150 mL water, add 25 mL 10 mM mercuric chloride solution, mix, adjust pH to 9.0 \pm 0.5 with 5 M NaOH, make up to 250 mL with water.)

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Nova-Pak C18

Mobile phase: MeCN:buffer 25:75 (Buffer was 4.969 g Na₂HPO₄, 8.969 g NaH₂PO₄·H₂O, and 2.482 g anhydrous sodium thiosulfate in 1 L water.)

Flow rate: 0.8

Injection volume: 80-100

Detector: UV 325

CHROMATOGRAM

Retention time: 7.6

Internal standard: penicillin V

OTHER SUBSTANCES

Extracted: penicillin G

Simultaneous: ampicillin, chloramphenicol

KEY WORDS

muscle; liver; kidney; derivatization; cow; SPE; penicillin V is IS

REFERENCE

Boison, J.O.; Salisbury, C.D.C.; Chan, W.; MacNeil, J.D. Determination of penicillin G residues in edible animal tissues by liquid chromatography. *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 497-501

ANNOTATED BIBLIOGRAPHY

Blanchflower, W.J.; Hewitt, S.A.; Kennedy, G. Confirmatory assay for the simultaneous determination of five penicillins in muscle, kidney and milk using liquid chromatography-electrospray mass spectrometry. *Analyt.*, **1994**, *119*, 2595-2601 [LC-MS; meat; LOD 5-100 ng/g; extracted cloxacillin, dicloxacillin, oxacillin, penicillin G; nafcillin (IS)]

Orford, C.D.; Perry, D.; Adlard, M.W. The determination of naturally produced penicillins and their biosynthetic precursors using pre-column derivatisation with dansylaziridine. *J.Liq.Chromatogr.*, **1991**, *14*, 2665-2684 [also penicillin G, penicillin K, penicillin X; LOD 1000 ng/mL; fluorescence detection]

Martín, J.; Méndez, R.; Negro, A. Effect of temperature on HPLC separations of penicillins. *J.Liq.Chromatogr.*, **1988**, *11*, 1707-1716 [also amoxicillin, ampicillin, cloxacillin, piperacillin, penicillin G; column temp 15-55]

Mopper, B. Liquid chromatographic determination of penicillin V potassium in tablets and powders for oral solution. *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 39-41

Möller, J.; Hiddessen, R.; Niehoff, J.; Schügerl, K. On-line high-performance liquid chromatography for monitoring fermentation processes for penicillin production. *Anal.Chim.Acta*, **1986**, *190*, 195-203 [simultaneous impurities]

Moats, W.A. Determination of penicillin G, penicillin V, and cloxacillin in milk by reversed-phase high-performance liquid chromatography. *J.Agric.Food Chem.*, **1983**, *31*, 880-883 [gradient; LOD 5 ppb; extracted cloxacillin, penicillin G]

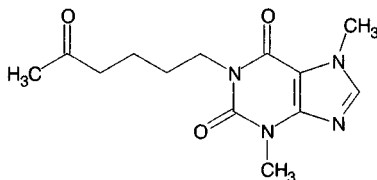
LeBelle, M.J.; Lauriault, G.; Wilson, W.L. High performance liquid chromatographic analysis of penicillin V solid dosage forms. *J.Liq.Chromatogr.*, **1980**, *3*, 1573-1578

Pentoxifylline

Molecular formula: C₁₃H₁₈N₄O₃

Molecular weight: 278.3

CAS Registry No.: 6493-05-6



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 100 μ L 20 μ g/mL tetracaine hydrochloride + 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 6 Shimpack CLS-ODS (Shimadzu)

Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 20:12:68

Column temperature: 40

Flow rate: 1.5

Injection volume: 20

Detector: UV 273

CHROMATOGRAM

Internal standard: tetracaine hydrochloride

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: Condition a 100 mg 1 mL Baker C18 SPE cartridge with 1 mL MeCN and 1 mL 60 mM pH 5.0 phosphate buffer. 500 μ L Plasma + 150 ng 7-(2'-chloroethyl)theophylline + 100 μ L 200 mM HCl + 400 μ L 60 mM pH 5.0 phosphate buffer (final pH 5.3), centrifuge at 10000 g for 3 min. Add the supernatant to the SPE cartridge, wash twice with 1 mL 60 mM pH 5.0 phosphate buffer, dry, elute three times with 200 μ L MeCN. Evaporate the eluate at 37° under a stream of air, reconstitute in 100 μ L mobile phase, vortex, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4 5 μ m LiChrospher 100 RP-18

Mobile phase: MeCN:dioxan:water 6.5:6.5:87, acidified to pH 3.0 with glacial acetic acid (0.5% v/v)

Flow rate: 1

Injection volume: 20

Detector: UV 275

CHROMATOGRAM

Retention time: 12.5

Internal standard: 7-(2'-chloroethyl)theophylline (11.2)

Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: caffeine, theobromine, theophylline

KEY WORDS

plasma; SPE

REFERENCE

Mancinelli, A.; Pace, S.; Marzo, A.; Martelli, E.A.; Passetti, G. Determination of pentoxifylline and its metabolites in human plasma by high-performance liquid chromatography with solid-phase extraction. *J.Chromatogr.*, **1992**, 575, 101-107

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 20 μ L 172 μ M acetophenone + 300 μ L MeOH, vortex, centrifuge at 1000 g for 5 min, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 5 μ m Hisep (Supelco)

Column: 150 \times 4.6 5 μ m Hisep (Supelco)

Mobile phase: MeCN:water 10:90

Injection volume: 50

Detector: UV 280

CHROMATOGRAM

Retention time: 3.3

Internal standard: acetophenone (4.5)

Limit of detection: 3.95 nM

KEY WORDS

plasma; rabbit; pharmacokinetics

REFERENCE

Lockemeyer, M.R.; Smith, C.V. Analysis of pentoxifylline in rabbit plasma using a Hisep high-performance liquid chromatography column. *J.Chromatogr.*, **1990**, 532, 162-167

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 20 μ L 0.5 mM IS in MeCN:water 22:78 + 100 μ L 3 M HCl + 5 mL dichloromethane:chloroform 1:1, mix on a rotary mixer at 20 rpm for 10 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature. Dissolve the residue in 200 μ L mobile phase, vortex, centrifuge at 1000 g for 1 min, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Zorbax ODS

Mobile phase: MeCN:water:acetic acid 22:77.9:0.1

Flow rate: 0.75

Injection volume: 50

Detector: UV 274

CHROMATOGRAM

Retention time: 8

Internal standard: 1-(6'-oxoheptyl)-3-dimethylxanthine (15)

Limit of detection: 53 nmol/L

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; also urine using SPE

REFERENCE

Lambert, W.E.; Yousouf, M.A.; Van Liedekerke, B.M.; De Roose, J.E.; De Leenheer, A.P. Simultaneous determination of pentoxifylline and three metabolites in biological fluids by liquid chromatography. *Clin.Chem.*, **1989**, *35*, 298-301

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Baker cyanopropyl SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 100 μ L 5 μ g/mL IS in water + 2 mL MeCN, mix, centrifuge at 600 g for 20 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute the residue in 1 mL water, add it to the SPE cartridge, wash with 200 μ L water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute in 1 mL mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 4 10 μ m LiChrosorb cyanopropyl

Column: 125 \times 4 5 μ m LiChrosorb cyanopropyl

Mobile phase: MeCN:water 1:99

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 8

Internal standard: 1-(6-oxoheptyl)-3,7-dimethylxanthine (9.5)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: caffeine, chlorthalidone, diclofenac, estriol, furosemide, phenacetin, phenobarbital, theobromine, theophylline, triamterene

Noninterfering: albuterol, amiloride, atenolol, mexiletine, tiapride

KEY WORDS

plasma; SPE

REFERENCE

Musch, G.; Hamoir, T.; Massart, D.L. Determination of pentoxifylline and its 5-hydroxy metabolite in human plasma by solid-phase extraction and high-performance liquid chromatography with ultra-violet detection. *J.Chromatogr.*, **1989**, *495*, 215-226

SAMPLE

Matrix: blood

Sample preparation: 250 μ L Whole blood + 4 mL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove a 3.7 mL aliquot of the supernatant and add it to 35 μ L 2 μ g/mL IS in MeOH, vortex for 10 s, filter (0.45 μ m PTFE), evaporate the filtrate to dry-

ness under a stream of nitrogen at 47°, reconstitute the residue in 2 mL hexane, vortex for 15 s, add 300 μ L water, vortex for 30 s, centrifuge at 1250 g for 15 min, inject a 250 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m LC-8-DB (Supelco)

Mobile phase: MeCN:water 24:76

Flow rate: 1

Injection volume: 250

Detector: UV 280

CHROMATOGRAM

Retention time: 6.4

Internal standard: 3-isobutyl-1-methylxanthine (7.6)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: antipyrine, caffeine, 8-chlorotheophylline, dimethyluric acid, dyphylline, hypoxanthine, methyluric acid, methylxanthine, pentifylline, phenacetin, 8-phenyltheophylline, theobromine, uric acid, xanthine

Interfering: pentobarbital

KEY WORDS

whole blood; human; rat

REFERENCE

Grasela, D.M.; Rocci, M.L., Jr. High-performance liquid chromatographic analysis of pentoxifylline and 1-(5'-hydroxyhexyl)-3,7-dimethylxanthine in whole blood. *J.Chromatogr.*, **1987**, *419*, 368-374

SAMPLE

Matrix: blood

Sample preparation: 250 μ L Plasma + 20 μ L 20 μ g/mL phenacetin in mobile phase, vortex for 15 s, add 2.5 mL MeCN, vortex for 15 s, centrifuge at 1980 g for 10 min. Remove the supernatant and filter (PTFE) it, evaporate the filtrate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 125 μ L water, vortex, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 5 μ m μ Bondapak C18

Mobile phase: MeCN:water 24:76

Flow rate: 1

Injection volume: 100

Detector: UV 280

CHROMATOGRAM

Retention time: 8.0

Internal standard: phenacetin (14.7)

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: aminopyrine, aspirin, caffeine, carbidopa, β -hydroxyethyltheophylline, β -hydroxypropyltheophylline, methicillin, methyldopa, nafcillin, nifedipine, penicillamine, penicillin V, penicillin G, phthalic acid, probenecid, salicylic acid, theobromine, theophylline

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Luke, D.R.; Rocci, M.L., Jr. Determination of pentoxifylline and a major metabolite, 3,7-dimethyl-1-(5'-hydroxyhexyl)xanthine, by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *374*, 191-195

SAMPLE

Matrix: blood

Sample preparation: Inject a 500 μ L aliquot of plasma onto column A and elute to waste with mobile phase A, after 4.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 5×4.6 25-40 μ m Lichroprep RP 2; B 60×4.6 7 μ m Nucleosil phenyl

Mobile phase: A 5 mM pH 7.1 Na_2HPO_4 ; B MeCN:5 mM pH 6.7 Na_2HPO_4 23:77

Column temperature: 45

Flow rate: A 1.8; B 0.5

Injection volume: 500

Detector: UV 273

CHROMATOGRAM

Retention time: 5.1

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; column-switching

REFERENCE

von Stetten, O.; Arnold, P.; Aumann, M.; Guserle, R. Direct measurement of pentoxifylline and its hydroxymetabolite from plasma using HPLC with column switching technique. *Chromatographia*, **1984**, *19*, 415-417

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150×6.5 μ m 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: 20

Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES

Simultaneous: caffeine, hypoxanthine, propentofylline, theobromine, theophylline, uric acid, xanthine

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: 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: 5.01 (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, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flvoxate, 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, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phentoin, pimizide, 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, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thietilperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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: 1 mL Urine 100 μ L 1 mg/mL IS in 10 mM NaOH, mix, add 6 mL dichloromethane, add 1 mL 1 M HCl, extract using a mechanical rotary inversion mixer at 20 rpm for 15 min, centrifuge at 2000 g for 5 min. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute the residue in 2 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 3.5 μ m Spherisorb-ODS 1

Mobile phase: MeOH:20 mM orthophosphoric acid 1:2.5, adjusted to pH 4 with 6 M NaOH

Flow rate: 1

Detector: UV 274

CHROMATOGRAM

Retention time: 18

Internal standard: 1-(3'-carboxypropyl)-3-methyl-7-propylxanthine (11.6)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pentoxifylline not usually seen in urine

REFERENCE

Bryce, T.A.; Burrows, J.L.; Jolley, K.W. Determination of 1-(3'-carboxypropyl)-3,7-dimethylxanthine and 1-(4'-carboxybutyl)-3,7-dimethylxanthine, two major metabolites of oxpentifylline, in urine by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, 344, 397–402

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Ostrovská, V.; Pechová, A.; Kováčová, D.; Svobodová, X.; Kusala, S. [Determination of pentoxifylline and its major metabolites in human plasma using HPLC]. *Cesk.Farm.*, **1990**, 39, 158–160

Garnier-Moiron, A.; Poirier, J.M.; Cheymol, G.; Jaillon, P. High-performance liquid chromatographic determination of pentoxifylline and its hydroxy metabolite in human plasma. *J.Chromatogr.*, **1987**, 416, 183–188

Rieck, W.; Platt, D. Determination of 3,7-dimethyl-1-(5-oxohexyl)-xanthine (pentoxifylline) and its 3,7-dimethyl-1-(5-hydroxyhexyl)-xanthine metabolite in the plasma of patients with multiple diseases using high-performance liquid chromatography. *J.Chromatogr.*, **1984**, 305, 419–427

Smith, R.V.; Yang, S.K.; Davis, P.J.; Bauza, M.T. Determination of pentoxifylline and its major metabolites in microbial extracts by thin-layer and high-performance liquid chromatography. *J.Chromatogr.*, **1983**, 281, 281–287

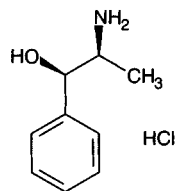
Chivers, D.A.; Birkett, D.J.; Miners, J.O. Simultaneous determination of pentoxifylline and its hydroxy metabolite in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1981**, 225, 261–265

Phenylpropanolamine

Molecular formula: C₉H₁₄ClNO

Molecular weight: 151.2

CAS Registry No.: 154-41-6 (phenylpropanolamine hydrochloride),
14838-15-4 (phenylpropanolamine)



SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μ L 1 M NaOH + 4 mL diethyl ether, extract, repeat extraction. Combine organic layers and evaporate them to dryness at 40° under a stream of nitrogen. Dissolve the residue in 300 μ L mobile phase A, inject a 200 μ L aliquot. Urine. 100 μ L Urine + 1 mL 100 mM NaOH + 4 mL diethyl ether, extract, repeat extraction. Combine organic layers and evaporate them to dryness at 40° under a stream of nitrogen. Dissolve the residue in 300 μ L mobile phase A, inject a 100 μ L aliquot. Inject onto column A and elute with mobile phase A, at an appropriate time (typically, 6.5 min) the effluent from column A was eluted onto column B using mobile phase A, when all compounds of interest had passed onto column B (typically, 8.7 min) elution of column B was continued with mobile phase B and the effluent from column B was monitored.

HPLC VARIABLES

Column: A 70 \times 4.6 5 μ m A type YMC ODS (Yamamura); B 100 \times 4.6 5 μ m A type YMC ODS (Yamamura)

Mobile phase: A MeCN:buffer 5:95 containing 5 mM sodium butanesulfonate; B MeCN:buffer 5:95 (Buffer was 20 mM KH₂PO₄ adjusted to pH 3.5 with 10% orthophosphoric acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 100-200

Detector: UV 205

CHROMATOGRAM

Retention time: 13

Limit of detection: 0.4 ng/mL (plasma); 8 ng/mL (urine)

KEY WORDS

plasma; column-switching; heart cut

REFERENCE

Yamashita, K.; Motohashi, M.; Yashiki, T. High-performance liquid chromatographic determination of phenylpropanolamine in human plasma and urine, using column switching combined with ion-pair chromatography. *J.Chromatogr.*, **1990**, 527, 103-114

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 10 mg/mL solution in 500 mM sodium bicarbonate solution, extract a 10 mL aliquot twice with 15 mL portions of dichloromethane. Combine the extracts and add 10 μ L phenylisothiocyanate, evaporate to dryness under a stream of air, reconstitute with 10 mL MeOH, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 70 \times 2.1 CO:PELL ODS

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeOH:water:acetic acid 45:54:1

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: ephedrine, lidocaine, pseudoephedrine

KEY WORDS

derivatization

REFERENCE

Nogge, F.T., Jr.; Clark, C.R. Liquid chromatographic analysis of samples containing cocaine, local anesthetics, and other amines. *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 151-157

SAMPLE

Matrix: formulations

Sample preparation: Grind ten tablets to a fine powder, add 15 mL MeCN:buffer 50:50, sonicate for 15 min, make up to 20 mL with MeCN:buffer 50:50, filter, dilute tenfold, inject a 10 μ L aliquot. (Buffer was 50 mM sodium perchlorate adjusted to pH 3.0 with 70% perchloric acid.)

HPLC VARIABLES

Column: 250 \times 4 10 μ m Lichrosorb RP-18

Mobile phase: Gradient. MeCN:buffer from 15:85 to 95:5 over 20 min, re-equilibrate at initial conditions for 10 min. (Buffer was 50 mM sodium perchlorate adjusted to pH 3.0 with 70% perchloric acid.)

Column temperature: 25

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Simultaneous: buzepide (UV 220), clocinizine

KEY WORDS

tablets

REFERENCE

Cavazzutti, G.; Gagliardi, L.; De Orsi, D.; Tonelli, D. Simultaneous determination of buzepide, phenylpropanolamine, and clocinizine on pharmaceutical preparations by ion-pair reversed-phase HPLC. *J.Liq.Chromatogr.*, **1995**, *18*, 227-234

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 μ m 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 260

CHROMATOGRAM

Retention time: 3.5

Limit of detection: 15.1 μg/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen (UV 310), caffeine (UV 298), chlorpheniramine (UV 265), guaifenesin (glycerylguaiacolate) (UV 284)

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 10 mL to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Whatman 10 μm PXS SCX

Mobile phase: MeOH:100 mM (NH₄)H₂PO₄, apparent pH 6.2

Column temperature: 40

Flow rate: 2

Injection volume: 20

Detector: UV 263

CHROMATOGRAM

Retention time: 3.12

OTHER SUBSTANCES

Simultaneous: 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: Tablets. One tablet + 50 mL MeOH, sonicate, make up to 100 mL with MeOH, centrifuge for 15 min. Remove 1 mL supernatant, make up to 10 mL with mobile phase, inject a 50 μL aliquot. Drops. Dilute drops with the mobile phase so that

the concentration of phenylpropanolamine hydrochloride is 50 µg/mL, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 Cyclobond I (Advanced Separation Technologies)

Mobile phase: MeOH:50 mM NaH₂PO₄ adjusted to pH 7.0 with 0.1 M NaOH 30:70

Column temperature: 35

Flow rate: 1.5

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

Simultaneous: pheniramine, pyrilamine (mepyramine)

KEY WORDS

tablets; drops

REFERENCE

el-Gizawy, S.M.; Ahmed, A. High-performance liquid chromatographic determination of mepyramine maleate, pheniramine maleate and phenylpropanolamine hydrochloride in tablets and drops. *Analyt.* **1987**, *112*, 867-869

SAMPLE

Matrix: formulations

Sample preparation: Injections. Dilute 1.5 mL of a 20 mg/mL injection to 100 mL with water, remove a 10 mL aliquot and add it to 8 mL 2% phenylpropanolamine hydrochloride, make up to 100 mL with water, inject a 20 µL aliquot. Tablets. Grind tablets to a fine powder, weigh out amount equivalent to about 10 mg hydralazine, mix thoroughly with 2 mL 500 mM HCl, make up to 100 mL with water, shake for 2-3 min, filter, discard first 15 mL. 15 mL Filtrate + 4 mL 2% phenylpropanolamine hydrochloride, make up to 50 mL with water, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18

Mobile phase: MeOH:15 mM KH₂PO₄:glacial acetic acid 2:97.9:0.1

Flow rate: 3

Injection volume: 20

Detector: UV 256

CHROMATOGRAM

Retention time: 3

Internal standard: phenylpropanolamine

OTHER SUBSTANCES

Simultaneous: hydralazine, hydrochlorothiazide

KEY WORDS

injections; tablets; phenylpropanolamine is IS

REFERENCE

Das Gupta, V. Quantitation of hydralazine hydrochloride in pharmaceutical dosage forms using high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1985**, *8*, 2497-2509

SAMPLE

Matrix: formulations

Sample preparation: Grind tablets to a fine powder and weigh out powder equivalent to about 80 mg metronidazole, add 90 mL water, heat to 60° with stirring, cool to room temperature, make up to 100 mL with water, filter, reject first 20 mL of filtrate. Mix 12.5 mL filtrate with 7 mL 10 mg/mL phenylpropanolamine hydrochloride in water, make up to 50 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak phenyl

Mobile phase: 20 mM KH_2PO_4 , pH 4.2

Flow rate: 2.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 3.5

Internal standard: phenylpropanolamine hydrochloride

OTHER SUBSTANCES

Simultaneous: metronidazole

Noninterfering: excipients, mannitol

KEY WORDS

tablets; stability-indicating; phenylpropanolamine is IS

REFERENCE

Das Gupta, V. Quantitation of metronidazole in pharmaceutical dosage forms using high-performance liquid chromatography. *J.Pharm.Sci.*, **1984**, 73, 1331-1333

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: 4.5

OTHER SUBSTANCES

Simultaneous: impurities, degradation products, benzoic acid, guaifenesin, phenylephrine

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

Sample preparation: Capsules and Tablets. Leach 1 g of ground capsule or tablet with 250 mL 0.4 mg/mL 2,5-dihydroxybenzoic acid in water, sonicate for 10 min, centrifuge at 2500 rpm for 5 min, inject an aliquot. Liquid formulations. Dilute 4-25 mL of the formulation to 250 mL with 0.4 mg/mL 2,5-dihydroxybenzoic acid in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 C8

Mobile phase: MeOH:water:PIC-B5 300:675:25 (PIC-B5 (Waters) is 200 mM sodium pentanesulfonate in glacial acetic acid.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 6

Internal standard: 2,5-dihydroxybenzoic acid (4.5)

OTHER SUBSTANCES

Simultaneous: impurities, degradation products, guaifenesin, phenylephrine

KEY WORDS

capsules; tablets; liquid formulations; stability-indicating

REFERENCE

Schieffer, G.W.; Hughes, D.E. Simultaneous stability-indicating determination of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and guaifenesin in dosage forms by reversed-phase paired-ion high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, *72*, 55-59

SAMPLE

Matrix: formulations

Sample preparation: Crush 10 tablets, add 250 mL 50 mM HCl in EtOH:water 50:50, heat for 15 min on a steam bath, shake mechanically for 2 h, filter (glass fiber GF/A, Whatman), inject a 30 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μ m Partisil-10-ODS

Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 \pm 0.02 with 1 M ammonium hydroxide.)

Flow rate: 3.8

Injection volume: 30

Detector: UV 216.5

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: apocopolamine, methscopolamine, pheniramine, pyrilamine, tropic acid

KEY WORDS

tablets

REFERENCE

Heidemann, D.R. High-pressure liquid chromatographic determination of methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate in tablets. *J.Pharm.Sci.*, **1981**, *70*, 820-822

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:** 4.5

OTHER SUBSTANCES**Simultaneous:** amphetamine, diethylpropion, ephedrine, fenfluramine, methamphetamine, phentermine**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 Ci-94, 1994-5, Rainin Instrument Co., Woburn MA, 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, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphe-

nesin, 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, fenopropfen, 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, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyriline, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelenamine, 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 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: 1.312

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, 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: Prepare a 0.5 mg/mL solution in water, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 4.9

OTHER SUBSTANCES

Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranlycypromine, tripeleennamine

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: Prepare a 1.5 mg/mL solution, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: Supelguard LC-8-DB (Supelco)

Column: 50 \times 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: 1

OTHER SUBSTANCES

Simultaneous: chlorpheniramine, methscopolamine, pseudoephedrine, triprolidine

REFERENCE

Supelco Catalog, Supelco, Inc., Bellefonte MA, 1992, p. 179

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 5:1.5:0.5:93

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: ephedrine, pseudoephedrine

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 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: 2.25

OTHER SUBSTANCES

Simultaneous: amphetamine, benzphetamine, benzylmorphine, bromo-STP, buprenorphine, caffeine, codeine, codeine-N-oxide, dextromoramide, dextropropoxyphene, diethylpropion, dihydrocodeine, dihydromorphine, dimethylamphetamine, ephedrine, ethoheptazine, ethylmorphine, etorphine, fenethyline, fenfluramine, fentanyl, hydrocodone, 4-hydroxyamphetamine, hydroxypethidine, levallorphan, levorphanol, mazindol, mephentermine, mescaline, methadone, methamphetamine, methylenedioxyamphetamine, methylephedrine, methylphenidate, morphine, morphine-3-glucuronide, morphine-N-oxide, nalorphine, naloxone, norcodeine, norlevorphanol, normetanephrine, normethadone, normorphine, norpethidine, norpipanone, noscapine, papaverine, pemoline, phenazocine, phendimetrazine, phenelzine, 2-phenethylamine, phenoperidine, phenylephrine, pholcodine, piritramide, prolintane, pseudoephedrine, STP, thebaine, tranlycypromine, trime-thoxyamphetamine, tyramine

Noninterfering: dopamine, levodopa, methyl dopa, methyl dopate, norepinephrine

Interfering: acetylcodeine, chlorphentermine, diamorphine, dipipanone, epinephrine, fen-camfamin, meperidine, monoacetylmorphine, norpseudoephedrine, oxycodone, pentazo-cine, phentermine, pipradol, thebacon

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: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μ m), inject 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 Lichrospher 5 μ m 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: 4

OTHER SUBSTANCES

Extracted: benzoylecgonine, cocaine, diphenhydramine, ephedrine, lidocaine, morphine, nordiazepam, norpropoxyphene, nortriptyline

Also analyzed: amitriptyline, amphetamine, codeine (different gradient), meperidine

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: 5 mL Urine + 25 μ L mobile phase + 100 μ L 10 M NaOH + 2 mL diethyl ether + 3 g sodium sulfate, shake for 20 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4 5 μ m LiChrospher 60 RP Select B

Mobile phase: 200 mM pH 5.5 phosphate buffer containing 150 mM triethylamine (Wash column with MeOH for 15 min and with water for 15 min at the end of each day.)

Column temperature: 40

Flow rate: 1.3

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 11.5

Internal standard: phenylpropanolamine

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: ephedrine, ethylephedrine, N-methylephedrine, norephedrine, norpseudoephedrine, pseudoephedrine

Noninterfering: amfepramone, amphetamine, caffeine, chlorphentermine, cocaine, codeine, cropropamide, crothetamide, dimethylamphetamine, etamivan, fencamfamine, heptaminol, leptazol, lidocaine, meperidine, methoxamine, methylamphetamine, methylphenidate, nicotine, niketamine, phendimetrazine, phenmetrazine, pipradol, procaine, prolintane, strychnine

KEY WORDS

phenylpropanolamine is IS

REFERENCE

Imaz, C.; Carreras, D.; Navajas, R.; Rodriguez, C.; Rodriguez, A.F.; Maynar, J.; Cortes, R. Determination of ephedrine in urine by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *631*, 201-205

SAMPLE

Matrix: urine

Sample preparation: 500 μ L Urine + chlorpheniramine + 100 μ L buffer, centrifuge at 11000 g for 30 s, inject a 500 μ L 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 μ L mobile phase B, with 200 μ L 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 start 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, 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 \times 2.1 12-20 μ m PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 \times 3.2 11 μ m Aminex A-28 (Bio-Rad); C 25 \times 3.2 5 μ m C8 (Phenomenex) + 150 \times 4.6 5 μ m 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: 40 (B, C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210; UV 235

CHROMATOGRAM

Retention time: k' 2.2

Internal standard: chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, benzoylecgonine, caffeine, codeine, cotinine, desipramine, diazepam, diphenhydramine, ephedrine, flurazepam, hydrocodone, hydromorphone, imipramine, lidocaine, methadone, methamphetamine, morphine, nordiazepam, nortriptyline, oxazepam, pentazocine, phenmetrazine, phenobarbital, secobarbital

Interfering: amphetamine, phentermine

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

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Doyle, T.D.; Brunner, C.A.; Vick, J.A. Enantiomeric analysis of phenylpropranolamine in plasma via resolution of dinitrophenylurea derivatives on a high performance liquid chromatographic chiral stationary phase. *Biomed.Chromatogr.*, **1991**, *5*, 43–46

Stockley, C.S.; Wing, L.M.; Miners, J.O. Stereospecific high-performance liquid chromatographic assay for the enantiomers of phenylpropranolamine in human plasma. *Ther.Drug Monit.*, **1991**, *13*, 332–338

Shi, R.J.Y.; Gee, W.L.; Williams, R.L.; Benet, L.Z.; Lin, E.T. Ion-pair liquid chromatographic analysis of phenylpropranolamine in plasma and urine by post-column derivatization with o-phthalaldehyde. *J.Liq.Chromatogr.*, **1985**, *8*, 1489–1500 [post-column reaction; fluorescence detection; LOD 2 ng/mL; α -methylbenzylamine (IS)]

Costanzo, S.J. Selection of mixed ion-pair modifiers for high-performance liquid chromatographic mobile phase. *J.Chromatogr.*, **1984**, *314*, 402–407 [simultaneous benzoic acid, guaifenesin, phenylephrine]

Das Gupta, V.; Heble, A.R. Quantitation of acetaminophen, chlorpheniramine maleate, dextromethorphan hydrobromide, and phenylpropranolamine hydrochloride in combination using high-performance liquid chromatography. *J.Pharm.Sci.*, **1984**, *73*, 1553–1556

Dye, D.; East, T.; Bayne, W.F. High-performance liquid chromatographic method for post-column, in-line derivatization with o-phthalaldehyde and fluorometric detection of phenylpropranolamine in human urine. *J.Chromatogr.*, **1984**, *284*, 457–461

Dowse, R.; Haigh, J.M.; Kanfer, I. Determination of phenylpropranolamine in serum and urine by high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, *72*, 1018–1020

Tan, H.S.; Salvador, G.C. Assay of mixtures of phenylpropranolamine hydrochloride and caffeine in appetite suppressant formulations by high-performance liquid chromatography. *J.Chromatogr.*, **1983**, *261*, 111–116

Lurie, I.S. Improved isocratic mobile phases for the reverse phase ion-pair chromatographic analysis of drugs of forensic interest. *J.Liq.Chromatogr.*, **1981**, *4*, 399–408 [also amobarbital, amphetamine, butabarbital, caffeine, diamorphine, ephedrine, methamphetamine, pentobarbital, phenobarbital, phentermine, secobarbital]

Lurie, I.S.; Demchuk, S.M. Optimization of a reverse phase ion-pair chromatographic separation for drugs of forensic interest. II. Factors effecting selectivity. *J.Liq.Chromatogr.*, **1981**, *4*, 357–374 [also acetaminophen, acetylcodeine, acetylmorphine, aminopyrene, aminopyrine, amobarbital, amphetamine, antipyrine, benzocaine, butabarbital, caffeine, cocaine, codeine, diamorphine, diazepam, diethylpropion, DMT, ephedrine, glutethimide, Lampa, lidocaine, LSD, MDA, mecloqualone, mescaline, methamphetamine, methapyrilene, methaqualone, methpyrilene, methylphenidate, morphine, narcotine, papaverine, PCP, pentobarbital, phenacyclidine, phendimetrazine, phenmetrazine, phenobarbital, phentermine, procaine, quinidine, quinine, secobarbital, strychnine, TCP, tetracaine, thebaine, theophylline]

Mason, W.D.; Amick, E.N. High-pressure liquid chromatographic analysis of phenylpropranolamine in human plasma following derivatization with o-phthalaldehyde. *J.Pharm.Sci.*, **1981**, *70*, 707–709

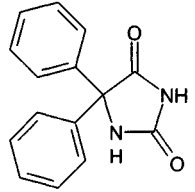
- Achari, R.G.; Jacob, J.T. A study of the retention behavior of some basic drug substances by ion-pair HPLC. *J.Liq.Chromatogr.*, **1980**, 3, 81–92 [also N-acetylprocainamide, antazoline, atropine, caffeine, chlorpheniramine, codeine, ephedrine, epinephrine, naphazoline, papaverine, pheniramine, phenylephrine, phenylpropanolamine, procainamide, quinidine, scopolamine, xylocaine]
- Muhammad, N.; Bodnar, J.A. Quantitative determination of guaifenesin, phenylpropanolamine hydrochloride, sodium benzoate & codeine phosphate in cough syrups by high-pressure liquid chromatography. *J.Liq.Chromatogr.*, **1980**, 3, 113–122

Phenytoin

Molecular formula: C₁₅H₁₂N₂O₂

Molecular weight: 252.3

CAS Registry No.: 57-41-0, 630-93-3 (sodium salt)



SAMPLE

Matrix: blood

Sample preparation: Add two volumes of MeCN to the mouse serum, mix, centrifuge at 1500 g for 5 min, inject a 5 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Sentry (Waters)

Column: 150 × 4.6 Nova-Pak C18

Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110

Column temperature: 40

Flow rate: 0.5

Injection volume: 5

Detector: UV 214

CHROMATOGRAM

Retention time: 9.5

OTHER SUBSTANCES

Extracted: carbamazepine, carbamazepine-10,11-epoxide, phenobarbital, phenylethyl malonamide, primidone

KEY WORDS

serum; mouse

REFERENCE

Capparella, M.; Foster, W., III; Larrousse, M.; Phillips, D.J.; Pomfret, A.; Tuvim, Y. Characteristics and applications of a new high-performance liquid chromatography guard column. *J.Chromatogr.A*, **1995**, *691*, 141–150

SAMPLE

Matrix: blood

Sample preparation: Plasma + methyl p-hydroxybenzoate in MeCN, centrifuge, mix the supernatant with an equal amount of water, inject an aliquot.

HPLC VARIABLES

Column: ODS-1

Mobile phase: MeCN:MeOH:10 mM pH 5.75 potassium phosphate buffer 25:5:70

Detector: UV 210

CHROMATOGRAM

Internal standard: methyl p-hydroxybenzoate

Limit of quantitation: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Fleishaker, J.C.; Pearson, L.K.; Peters, G.R. Phenytoin causes a rapid increase in 6 β -hydroxycortisol urinary excretion in humans—A putative measure of CYP3A induction. *J.Pharm.Sci.*, **1995**, *84*, 292–294

SAMPLE

Matrix: blood

Sample preparation: Centrifuge serum at 15600 g for 3 min, inject a 400 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 5 μ m GFF-II-S5-80 glycine-phenylalanine-phenylalanine internal surface bonded phase (Regis)

Column: 150 \times 4.6 5 μ m GFF-II-S5-80 glycine-phenylalanine-phenylalanine internal surface bonded phase (Regis)

Mobile phase: THF: 12.5 mM pH 7.4 phosphate buffer 1:99

Column temperature: 37

Flow rate: 1

Injection volume: 400

Detector: UV 254

CHROMATOGRAM

Retention time: 17.5 (free phenytoin), 14.5 (bound phenytoin)

KEY WORDS

serum; direct injection

REFERENCE

Gurley, B.J.; Marx, M.; Olsen, K. Phenytoin free fraction determination: comparison of an improved direct serum injection high-performance liquid chromatographic method to ultrafiltration coupled with fluorescence polarization immunoassay. *J.Chromatogr.B*, **1995**, *670*, 358–364

SAMPLE

Matrix: blood

Sample preparation: Filter (0.22 μ m), inject a 5 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 4 5 μ m LiChrospher 100 Diol

Mobile phase: MeCN:50 mM pH 6.9 phosphate buffer 12:88

Flow rate: 0.6

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital

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: 50 μ L Plasma + 100 μ L MeCN, centrifuge, inject a 5 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 100 \times 4.6 3.5 μ m Zorbax SB

Mobile phase: MeCN:MeOH:10 mM pH 7.1 phosphate buffer 7:34:59

Flow rate: 1.5

Injection volume: 5

Detector: UV 220

CHROMATOGRAM

Limit of detection: <1 μ M

OTHER SUBSTANCES

Extracted: carbamazepine, carbamazepine epoxide, hydroxycarbamazepine, lamotrigine, oxcarbazepine, phenobarbital

Also analyzed: ibuprofen, naproxen, trimethoprim

KEY WORDS

plasma

REFERENCE

Svensson, J.O. Simple HPLC method for determination of antiepileptic drugs in plasma (Abstract 102). *Ther.Drug Monit.*, **1995**, *17*, 408

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 25 μ L IS solution + 50 μ L 200 mM 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m TSKgel ODS-80TM (Tosoh)

Mobile phase: MeCN:8 mM NaH₂PO₄ 35:65

Flow rate: 0.8

Injection volume: 50

Detector: UV 215

CHROMATOGRAM

Retention time: 16

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; rat; pharmacokinetics

REFERENCE

Tanaka, E.; Ishikawa, A.; Misawa, S. Changes in the metabolism of three model substrates catalysed by different P450 isozymes when administered as a cocktail to the carbon tetrachloride-intoxicated rat. *Xenobiotica*, **1995**, *25*, 1111–1118

SAMPLE**Matrix:** blood**Sample preparation:** 100 μ L Serum + 50 μ L 10 μ g/mL IS + 100 μ L 200 mM 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m TSKgel ODS 80TM (Tosoh)**Mobile phase:** MeCN:8 mM pH 6 NaH₂PO₄ 35:65**Flow rate:** 0.8**Injection volume:** 50**Detector:** UV 215

CHROMATOGRAM**Retention time:** 16**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (24.7)**Limit of quantitation:** 50 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites**Simultaneous:** carbamazepine, diazepam, ethosuximide, nitrazepam, oxazolam, phenobarbital, primidone

KEY WORDS

rat; serum; pharmacokinetics

REFERENCETanaka, E.; Sakamoto, N.; Inubushi, M.; Misawa, S. Simultaneous determination of plasma phenytoin and its primary hydroxylated metabolites in carbon tetrachloride-intoxicated rats by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 673, 147–151

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Serum + 600 μ L allobarbitol in 75 mM pH 6.8 phosphate buffer, add 200 units β -glucuronidase, heat at 37° for 30 min, add 1 mL of this solution to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 2.5 mL MTBE. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μ L MeOH:water 50:50, inject a 10 μ L aliquot onto columns A and B in series and elute with mobile phase A. After 12 min elute column A with mobile phase B, continue to elute column B with mobile phase A. Carbamazepine diol, carbamazepine epoxide, phenytoin, and carbamazepine elute from column A and the enantiomers of 5-(p-hydroxyphenyl)-5-phenylhydantoin and mephobarbital, phenobarbital, zonisamide, and allobarbitol elute from column B. Re-equilibrate columns A and B with mobile phase A for 5 min before the next injection.

HPLC VARIABLES**Column:** A 250 \times 4 4 μ m Superspher RP-18e (E. Merck); B 250 \times 4 4 μ m Superspher RP-18e (E. Merck)**Mobile phase:** A MeOH:11.2 mM β -cyclodextrin in 20 mM KH₂PO₄ 5:95; B MeCN:20 mM KH₂PO₄ 16:84**Flow rate:** 0.8**Injection volume:** 10**Detector:** UV 210 (A); UV 210 (B)

CHROMATOGRAM**Retention time:** 19 (column A)

Internal standard: allobarbital

Limit of detection: 3.9 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine, carbamazepine diol, carbamazepine epoxide, 5-(p-hydroxyphenyl)-5-phenylhydantoin, mephobarbital, phenobarbital, zonisamide

KEY WORDS

serum; column-switching; SPE

REFERENCE

Eto, S.; Noda, H.; Noda, A. Simultaneous determination of antiepileptic drugs and their metabolites, including chiral compounds, via β -cyclodextrin inclusion complexes by a column-switching chromatographic technique. *J.Chromatogr.B*, **1994**, *658*, 385–390

SAMPLE

Matrix: blood

Sample preparation: Inject sample onto column A with mobile phase A and elute for 3 min. Backflush contents of column A onto column B with mobile phase B for 6 min and elute column B with mobile phase B and monitor eluant.

HPLC VARIABLES

Column: A 10 \times 3 BioTrap Acid C18 (ChromTech); B 10 \times 3 CT-sil C18 guard column + 150 \times 4.6 5 μ m CT-sil C18 (ChromTech)

Mobile phase: A 82 mM pH 6.0 phosphate buffer; B MeCN:82 mM pH 6.0 phosphate buffer 50:50

Flow rate: A 0.55; B 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 10.5

Limit of quantitation: 4.56 μ g/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine

KEY WORDS

plasma; column-switching; direct injection

REFERENCE

Hermansson, J.; Grahn, A. Determination of drugs by direct injection of plasma into a biocompatible extraction column based on a protein-entrapped hydrophobic phase. *J.Chromatogr.A*, **1994**, *660*, 119–129

SAMPLE

Matrix: blood

Sample preparation: Condition an Extrashot-Silica (diatomaceous earth) SPE cartridge (Kusano Scientific) with 200 μ L EtOH and 200 μ L dichloromethane, force out the remaining solvent with 500 μ L air. Add 5 μ L serum to the surface of the cartridge and pass 130 μ L dichloromethane gently through the cartridge into the 100 μ L sample loop.

HPLC VARIABLES

Column: 125 \times 4 5 μ m LiChrosorb Si60

Mobile phase: n-Hexane:dichloromethane:EtOH:acetic acid 82.8:15:2:0.2

Flow rate: 1
Injection volume: 100
Detector: UV 240

CHROMATOGRAM

Retention time: 7.9
Limit of quantitation: 5 µg/mL

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital

KEY WORDS

serum; normal phase; SPE

REFERENCE

Kouno, Y.; Ishikura, C.; Homma, M.; Oka, K. Simple and accurate high-performance liquid chromatographic method for the measurement of three antiepileptics in therapeutic drug monitoring. *J.Chromatogr.*, **1993**, 622, 47-52

SAMPLE

Matrix: blood
Sample preparation: 100 µL Serum + 200 µL 20 µg/mL butalbital in MeCN, vortex, centrifuge 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 LiChroSpher RP-8 5 µm
Mobile phase: MeCN:water:100 mM pH 7.0 phosphate buffer 20:75:5
Column temperature: 45
Flow rate: 2
Injection volume: 50
Detector: UV 212

CHROMATOGRAM

Retention time: 10.6
Internal standard: butalbital (3.8)

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital
Simultaneous: 1

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: Inject 20 µL serum onto column A with mobile phase A and elute to waste, after 1.5 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 5 μm Nucleosil C18

Mobile phase: A 14 mM NaH₂PO₄ containing 6 mM Na₂HPO₄; B MeCN:MeOH:14 mM NaH₂PO₄ containing 6 mM Na₂HPO₄ 15:20:65

Flow rate: 0.8

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 26

Limit of detection: 1 μg/mL

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital, primidone

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: 500 μL Plasma + 500 μL 1 M pH 5.0 sodium acetate buffer + 50 μL 210 μg/mL tolybarbital in MeOH, vortex for 15 s, add 4 mL dichloromethane:ethyl acetate 2:1, shake for 5 min, repeat extraction. Combine the organic layers and evaporate them 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: 250 × 4.6 5 μm Spherisorb Octyl C8

Mobile phase: MeOH:MeCN:THF:10 mM pH 6.5 ammonium phosphate buffer 16:11:7:66

Column temperature: 40

Flow rate: 1.5

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 11.6

Internal standard: tolybarbital (6.7)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine, carbamazepine-10,11-epoxide, carbamazepinediol, cyheptamide, felbamate, 5-(p-hydroxyphenyl)-5-phenylhydantoin

Also analyzed: ethosuximide, ethotoin, lorazepam, phenobarbital, phenylethylmalonamide, primidone

KEY WORDS

plasma

REFERENCE

Rommel, R.P.; Miller, S.A.; Graves, N.M. Simultaneous assay of felbamate plus carbamazepine, phenytoin, and their metabolites by liquid chromatography with mobile phase optimization. *Ther. Drug Monit.*, **1990**, *12*, 90-96

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 μ L plasma then 50 μ L 10 μ g/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 μ L mobile phase, inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil-LC-8

Mobile phase: MeCN:water 20:80

Flow rate: 3.3

Injection volume: 15

Detector: UV 208

CHROMATOGRAM

Retention time: 12.54

Internal standard: tolylphenobarbital (7.57)

Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: amobarbital, barbital, butabarbital, caffeine, carbamazepine, carbamazepine diol, carbamazepine epoxide, chloramphenicol, ethosuximide, glutethimide, mephenytoin, methaqualone, methyprylon, nirvanol, pentobarbital, phenacetamide, phenobarbital, primidone, secobarbital, theophylline

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, p-hydroxyphenobarbital, imipramine, lidocaine, methotrexate, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, 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: 100 μ L Serum + 100 μ L 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 μ L mobile phase, inject a 6-10 μ L 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 \times 4.6 Supelguard LC-1 (Supelco)

Column: 250 \times 4.6 5 μ m 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: 6.28

Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, primidone, secobarbital, theophylline, thiopental

Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphyllyne, 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: 100 μL Plasma + 200 μL 1 M HCl saturated with ammonium sulfate, vortex for 20 s, add 60 μL 10 $\mu\text{g}/\text{mL}$ 4-methylprimidone in MeCN, vortex for 20 s, centrifuge at 2700 g for 5 min, inject a 5-10 μL aliquot of the MeCN layer.

HPLC VARIABLES

Column: 250 \times 4.5 μm LiChrosorb RP-18

Mobile phase: MeOH:THF:50 mM pH 5.9 phosphate buffer 44:1:55

Column temperature: 50

Flow rate: 1.1

Injection volume: 5-10

Detector: UV 210

CHROMATOGRAM

Retention time: 8

Internal standard: 4-methylprimidone (5)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital, primidone, valproic acid

Simultaneous: acetaminophen, caffeine, chloramphenicol, diazepam, ethosuximide, ethylphenylmalonamide, glutethimide, lidocaine, methylphenobarbital, pentobarbital, salicylic acid, theophylline

KEY WORDS

plasma

REFERENCE

Kushida, K.; Ishizaki, T. Concurrent determination of valproic acid with other antiepileptic drugs by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, 338, 131-139

SAMPLE**Matrix:** blood**Sample preparation:** 200 μ L Serum + 1 mL 5 μ g/mL 5-tolyl-5-phenylhydantoin in MeCN, agitate for 3 min. Remove the supernatant and evaporate it to dryness, dissolve the residue in 1 mL mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES**Guard column:** pellicular reversed phase (Chrompack 28653)**Column:** 100 \times 3 octyl CP-tm-Spher C8 glass column (Chrompack)**Mobile phase:** MeCN:50 mM NaH₂PO₄ 30:70 adjusted to pH 2.2 with phosphoric acid**Flow rate:** 0.9**Injection volume:** 50**Detector:** UV 210

CHROMATOGRAM**Retention time:** 6**Internal standard:** 5-tolyl-5-phenylhydantoin (11)**Limit of detection:** 1 μ g/mL

OTHER SUBSTANCES**Extracted:** phenobarbital

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:** 50 μ L Serum + 50 μ L 10 μ g/mL IS in MeCN, vortex for 10 s, centrifuge at 3000 g for 1 min, remove the supernatant and place it in another tube, centrifuge for 1 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** 100 \times 8 5 μ m Nova Pak C18 Radial pak**Mobile phase:** MeCN:MeOH:acetone:buffer 8:21:10:61, adjusted to pH 7.95 \pm 0.02 with NaOH (Buffer was 1.36 g/L KH₂PO₄.)**Flow rate:** 2.8**Injection volume:** 20**Detector:** UV 200

CHROMATOGRAM**Retention time:** 7.70**Internal standard:** tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (4.89)**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, carbamazepine, ethosuximide, phenobarbital, primidone

Simultaneous: acetaminophen, N-acetylprocainamide, aspirin, ampicillin, caffeine, cephalirin, chloramphenicol, digoxin, disopyramide, hexobarbital, indomethacin, lidocaine, mephobarbital, methsuximide, nafcillin, pentobarbital, phenylethylmalonamide, procainamide, quinidine, salicylic acid, secobarbital, sulfamerazine, sulfamethazine, terbutaline, tetracycline, theobromine, theophylline

Noninterfering: acetazolamide, amikacin, cephalosporin C, gentamicin, propranolol, sulfadiazine, sulfamethoxazole, sulfisoxazole, tobramycin, valproic acid, verapamil

KEY WORDS

serum

REFERENCE

Ou, C.-N.; Rognerud, C.L. Simultaneous measurement of ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine, and their bioactive metabolites by liquid chromatography. *Clin. Chem.*, **1984**, *30*, 1667-1670

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 50 μ L 7 μ g/mL IS in water + 1 mL buffer, vortex for 10 s, add 5 mL n-hexane:ether:n-propanol 49:49:2, shake gently for 20 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μ L mobile phase, inject a 50-100 μ L aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Partisil 5 ODS-3

Mobile phase: MeCN:buffer 28:72 (Buffer was 300 μ L 1 M KH_2PO_4 and 50 μ L 900 mM phosphoric acid in 1.8 L water, pH 4.4.)

Column temperature: 50

Flow rate: 2.8

Injection volume: 50-100

Detector: UV 195

CHROMATOGRAM

Retention time: 7.8

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)

OTHER SUBSTANCES

Extracted: ethosuximide, secobarbital

Simultaneous: mephobarbital, paramethadione, phenobarbital, primidone

Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid

Interfering: carbamazepine

KEY WORDS

serum

REFERENCE

Levine, H.L.; Cohen, M.E.; Duffner, P.K.; Kustas, K.A.; Shen, D.D. An improved high-pressure liquid chromatographic assay for secobarbital in serum. *J.Pharm.Sci.*, **1982**, *71*, 1281-1283

SAMPLE

Matrix: blood

Sample preparation: 400 μ L Serum or plasma + 400 μ L 10 μ g/mL IS in acetone, vortex for 10 s, centrifuge at 4500-5000 g for 1 min, remove the supernatant to another tube, centrifuge for 30 s, inject a 5-7.5 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:MeOH:buffer 17:28:55, final pH 6.8-7.0 (Buffer was 400 μ L 1 M KH_2PO_4 in 1 L water, pH adjusted to 6.0 with 900 mM phosphoric acid.)

Column temperature: 30

Flow rate: 0.7

Injection volume: 5-7.5

Detector: UV 195

CHROMATOGRAM

Retention time: 16.1

Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (13.8)

OTHER SUBSTANCES

Extracted: carbamazepine, N-desmethylnmethsuximide, ethosuximide, phenobarbital, primidone

Simultaneous: acetaminophen, butalbital, caffeine, hexobarbital, methsuximide, phenacetin, phenylethylmalonamide, salicylic acid

KEY WORDS

serum; plasma

REFERENCE

Szabo, G.K.; Browne, T.R. Improved isocratic liquid-chromatographic simultaneous measurement of phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, and N-desmethylnmethsuximide in serum. *Clin.Chem.*, **1982**, *28*, 100-104

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 200 μ L 50 μ g/mL hexobarbital in MeCN + 25 μ L glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: μ Bondapak 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: 24.0

Internal standard: hexobarbital (20.6)

Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, 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: 500 μ L Plasma + 100 μ L heptabarbital in MeOH + 500 μ L 400 mM pH 7.0 sodium phosphate buffer + 10 mL ethyl acetate, extract. Evaporate the extract to dryness at 50°, reconstitute the residue in 20 μ L MeOH, inject a 3 μ L aliquot.

HPLC VARIABLES

Guard column: 50 \times 2.1 Whatman Co:Pell ODS

Column: 125 \times 4.5 5 μ m SAS Hypersil

Mobile phase: MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 7.5 with phosphoric acid.)

Flow rate: 1.6

Injection volume: 3

Detector: UV 200

CHROMATOGRAM

Retention time: 18.6

Internal standard: heptabarbital (9.8)

Limit of quantitation: 5.2 μ M

OTHER SUBSTANCES

Extracted: carbamazepine, ethosuximide, pheneturide, phenobarbital, primidone

Simultaneous: amobarbital, barbital, butabarbital, cyclobarbital, ethotoin, ethylphenacemide, glutethimide, methsuximide, pentobarbital, phenylethylmalonamide, secobarbital, sulfamethoxazole, sulthiame

KEY WORDS

plasma; horse

REFERENCE

Christofides, J.A.; Fry, D.E. Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography. *Clin.Chem.*, **1980**, *26*, 499-501

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum or plasma + 200 μ L 20 μ g/mL IS in MeOH:water 10:90 + 75 μ L glacial acetic acid, vortex for 30 s, add 5 mL chloroform, shake for 5 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 200 μ L mobile phase, inject a 40 μ L aliquot.

HPLC VARIABLES

Guard column: 30 \times 2.1 Permaphase ETH (DuPont)

Column: 250 \times 4.6 CLC 1 C8 (DuPont)

Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM KH_2PO_4 and 1 mM K_2HPO_4 adjusted to pH 5.6.)

Column temperature: 25

Flow rate: 2

Injection volume: 40

Detector: UV 220

CHROMATOGRAM

Retention time: 6.3

Internal standard: alphenal (5-allyl-5-phenylbarbituric acid) (4.4)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine, ethosuximide, phenobarbital, primidone

Simultaneous: amobarbital, barbital, chlordiazepoxide, codeine, ethotoin, glutethimide, hexobarbital, hydrocortisone, mephenytoin, mephobarbital, metharbital, methsuximide, nitrazepam, phenacetin, phensuximide, secobarbital

Noninterfering: acetaminophen, acetazolamide, amphetamine, bilirubin, caffeine, diazepam, dimenhydrinate, meperidine, meprobamate, methamphetamine, methaqualone, methylphenidate, nicotine, propoxyphene, theophylline, valproic acid

Interfering: pentobarbital

KEY WORDS

serum; plasma

REFERENCE

Rydzewski, R.S.; Gadsden, R.H.; Phelps, C.A. Simultaneous rapid HPLC determination of anticonvulsant drugs in plasma and correlation with EMIT. *Ann. Clin. Lab. Sci.*, **1980**, *10*, 89-94

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 \times 2.1 40 μm preparative grade C18 (Analytichem); B 250 \times 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: 11.90

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

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

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, dialysate

Sample preparation: Dialyze 400 μL plasma against 175 μL acceptor solution through a Cuprophane membrane (15 kDa cut-off) at 37° for 10 min, inject 500 μL acceptor solution (including the portion used for dialysis) onto column A at 0.71 mL/min, elute the contents of column A onto column B with mobile phase, remove column A from circuit and condition it with 1 mL acceptor solution, elute column B with mobile phase and monitor the effluent. Flush acceptor channel with 5 mL acceptor solution and plasma channel with 8 mL acceptor solution containing 25 $\mu\text{g}/\text{mL}$ Triton X-100. (Acceptor solution contained 5.9 g NaCl, 4.1 g sodium acetate, 0.3 g KCl, and 1.65 g sodium citrate in 1 L water, adjusted to pH 7.4 with citric acid.)

HPLC VARIABLES

Column: A 5 \times 1.6 Hypersil ODS-2; B 100 \times 3 5 μm Spherisorb ODS-2

Mobile phase: MeCN:THF:20 mM pH 6.0 phosphate buffer 22:6.5:71.5

Column temperature: 37

Flow rate: 0.6

Detector: UV 240

CHROMATOGRAM

Retention time: 6

Limit of detection: 800 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital

KEY WORDS

plasma; column-switching; dialysis

REFERENCE

Johansen, K.; Krogh, M.; Andresen, A.T.; Christophersen, A.S.; Lehne, G.; Rasmussen, K.E. Automated analysis of free and total concentrations of three antiepileptic drugs in plasma with on-line dialysis and high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 669, 281–288

SAMPLE

Matrix: blood, dialysate

Sample preparation: Dialyze 400 μL plasma against 175 μL acceptor solution through a 4 cm^2 cuprophan (cellulose acetate, molecular mass cut-off 15000) membrane for 25 min at 20° and 10 min at 37°, inject 500 μL acceptor solution (including the dialysis sample) onto column A at 0.5 mL/min, elute column A onto column B with mobile phase, remove column A from circuit and condition it with 1 mL acceptor solution, elute column B with mobile phase and monitor the effluent. Flush acceptor channel with 5 mL acceptor solution and plasma channel with 8 mL acceptor solution containing 50 $\mu\text{g}/\text{mL}$ Triton X-100. (Acceptor solution contained 5.9 g NaCl, 4.1 g sodium acetate, 0.3 g KCl, and 1.65 g sodium citrate in 1 L water, adjusted to pH 7.4 with citric acid.)

HPLC VARIABLES

Column: A 10 × 2 36 μm polystyrene-divinylbenzene (Dyno Particles); B 100 × 3 5 μm Spherisorb ODS-2

Mobile phase: MeCN:THF:20 mM pH 5 phosphate buffer 25:7.5:67.5

Column temperature: 37

Flow rate: 0.7

Detector: UV 220

CHROMATOGRAM

Retention time: 5

Limit of detection: 600 ng/mL

OTHER SUBSTANCES

Noninterfering: carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, valproic acid

KEY WORDS

plasma; column-switching; dialysis

REFERENCE

Andresen, A.T.; Rasmussen, K.E.; Rugstad, H.E. Automated determination of free phenytoin in human plasma with on-line equilibrium dialysis and column-switching high-performance liquid chromatography. *J.Chromatogr.*, **1993**, 621, 189-198

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Serum. 100 μL Serum + 200 μL MeCN, vortex for 10 s, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Saliva. 250 μL Saliva + 50 μL MeCN, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Urine. Condition a Sep-Pak SPE cartridge with 5 mL MeCN then 20 mL water. Add 2 mL urine to the cartridge, wash with 20 mL water, elute with 500 μL MeCN, inject 2 μL of the eluent.

HPLC VARIABLES

Guard column: 20 × 2 3 μm ODS-Hypersil

Column: 250 × 2 3 μm ODS-Hypersil

Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110

Column temperature: 40

Flow rate: 0.2

Injection volume: 2

Detector: UV 200

CHROMATOGRAM

Retention time: 11.6

Limit of quantitation: 780 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine, carbamazepine-10,11-epoxide, clonazepam, dihydrodihydroxycarbamazepine, hexobarbital, p-hydroxyphenobarbital, 5-(m-hydroxyphenyl)-5-phenylhydantoin, 5-(p-hydroxyphenyl)-5-phenylhydantoin, nitrazepam, phenobarbital, phenylethylmaleimide, primidone

Noninterfering: chlordiazepoxide, cyheptamide, diazepam, lorazepam, nordiazepam, oxazepam, prazepam, temazepam

KEY WORDS

serum; SPE

REFERENCE

Liu, H.; Delgado, M.; Forman, L.J.; Eggers, C.M.; Montoya, J.L. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. *J.Chromatogr.*, **1993**, *616*, 105-115

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize 20-200 mg brain tissue with 1 mL 1.5 µg/mL IS in 1% ammonium acetate buffer containing 1% sodium azide:MeCN 99:1, flush apparatus with 1 mL extraction buffer, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 µL MeOH, add 50 µL water, inject a 10-25 µL aliquot. Serum. 100 µL Serum + 1 mL 1.5 µg/mL IS in 1% ammonium acetate buffer containing 1% sodium azide:MeCN 99:1, mix, add 1 mL extraction buffer, mix, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 µL MeOH, add 50 µL water, inject a 10-25 µL aliquot. (Extraction buffer was 20 g NaH₂PO₄·2H₂O + 4.5 g Na₂HPO₄·2H₂O + 1.5 g sodium azide in 1 L water, pH 6. Extraction solvent was dichloromethane:isopropanol 97:3. Caution! Sodium azide is highly toxic!)

HPLC VARIABLES

Column: 200 × 2.1 5 µm Hypersil ODS

Mobile phase: Gradient. A was MeCN:50 mM (NH₄)H₂PO₄ (pH 4.4) 10:90. B was MeCN:50 mM (NH₄)H₂PO₄ (pH 4.4) 60:40. A:B from 85:15 to 55:45 over 9.5 min, keep at 55:45 for 0.5 min, return to 85:15 over 0.5 min.

Column temperature: 65

Flow rate: 0.3

Injection volume: 10-25

Detector: UV 207

CHROMATOGRAM

Retention time: 10.18

Internal standard: 5-ethyl-5-(p-tolyl)barbituric acid (9.07)

Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine, carbamazepine-10,11-epoxide, N-desmethylnethsuximide, phenobarbital, primidone

KEY WORDS

serum; SPE; brain

REFERENCE

Juergens, U.; Rambeck, B. Sensitive analysis of antiepileptic drugs in very small portions of human brain by microbore HPLC. *J.Liq.Chromatogr.*, **1987**, *10*, 1847-1863

SAMPLE

Matrix: blood, urine

Sample preparation: 100 µL Plasma or urine + 100 µL 20000 U/mL β-glucuronidase in 200 mM pH 4.9 sodium acetate buffer, heat at 37° for 20 h, add 20 µL 100 µg/mL mephenytoin, add 2 volumes of ethyl acetate, extract, centrifuge at 1000 g for 5 min, repeat extraction four more times. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 20 µL MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm RP C18 (Beckman)

Mobile phase: Gradient. Isopropanol:water 20:80, to 25:75 after 7 min (step gradient).

Flow rate: 1.4

Injection volume: 5

Detector: UV 225

CHROMATOGRAM

Retention time: 15

Internal standard: mephenytoin (8)

Limit of detection: 5 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

mouse; plasma

REFERENCE

Lum, J.T.; Vassanji, N.A.; Wells, P.G. Analysis of the toxicologically relevant metabolites of phenytoin in biological samples by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, *338*, 242–248

SAMPLE

Matrix: cell suspensions

Sample preparation: Centrifuge cell suspensions, inject a 200 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.5 5 μm C18 (IBM)

Mobile phase: MeCN:5 mM pH 3.0 phosphate buffer 27:73

Flow rate: 1.2

Injection volume: 200

Detector: UV 210

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Mays, D.C.; Pawluk, L.J.; Apseloff, G.; Davis, W.B.; She, Z.W.; Sagone, A.L.; Gerber, N. Metabolism of phenytoin and covalent binding of reactive intermediates in activated human neutrophils. *Biochem.Pharmacol.*, **1995**, *50*, 367–380

SAMPLE

Matrix: enzyme preparations

Sample preparation: 200 μL Enzyme preparation + 50 μL 20% trichloroacetic acid, vortex, centrifuge at 14000 rpm for 12 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 30 × 3.2 5 μm Hypersil C18

Column: 250 × 3.2 5 μm Hypersil C18

Mobile phase: MeOH:50 mM pH 6.5 ammonium phosphate 35:65

Column temperature: 40
Flow rate: 0.8
Injection volume: 100
Detector: UV 214

OTHER SUBSTANCES

Extracted: fosphenytoin

REFERENCE

TenHoor, C.N.; Stewart, B.H. Reconversion of fosphenytoin in the presence of intestinal alkaline phosphatase. *Pharm.Res.*, **1995**, *12*, 1806–1809

SAMPLE

Matrix: formulations

Sample preparation: Dissolve tablet in 10 mM HCl containing 90 mM KCl (pH 2.0), inject an aliquot.

HPLC VARIABLES

Column: 50 mm long ODS Hypersil C18

Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 32:68 containing 5 mM tetrabutylammonium

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 8.4

OTHER SUBSTANCES

Simultaneous: naproxen

KEY WORDS

tablets

REFERENCE

Neervannan, S.; Dias, L.S.; Southard, M.Z.; Stella, V.J. A convective-diffusion model for dissolution of two non-interacting drug mixtures from co-compressed slabs under laminar hydrodynamic conditions. *Pharm.Res.*, **1994**, *11*, 1288–1295

SAMPLE

Matrix: formulations

Sample preparation: Dissolve in water with rotating paddle, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 Zorbax ODS

Mobile phase: MeCN:water 40:60

Flow rate: 1.5

Detector: UV 200

KEY WORDS

oral dosage

REFERENCE

Watanabe, A.; Hanawa, T.; Sugihara, M.; Yamamoto, K. Release profiles of phenytoin from new oral dosage form for the elderly. *Chem.Pharm.Bull.*, **1994**, *42*, 1642–1645

SAMPLE

Matrix: microsomal incubations

Sample preparation: 2 mL Microsomal incubation + 25 μ L 1 mM IS in EtOH + 5 mL MTBE, rotate for 20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 300 μ L 0.05% orthophosphoric acid, vortex, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: Spherisorb 5 ODS

Mobile phase: MeCN:0.05% orthophosphoric acid 27:73

Flow rate: 1

Injection volume: 100

Detector: UV 214

CHROMATOGRAM

Retention time: 24

Internal standard: 5-(4'-methylphenyl)-5-phenylhydantoin (42)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver

REFERENCE

Ashforth, E.I.L.; Carlile, D.J.; Chenery, R.; Houston, J.B. Prediction of *in vivo* disposition from *in vitro* systems: Clearance of phenytoin and tolbutamide using rat hepatic microsomal and hepatocyte data. *J.Pharm.Exp.Ther.*, **1995**, 274, 761–766

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Spherisorb ODS-2

Mobile phase: MeCN:water 40:60

Flow rate: 0.8

Detector: UV 228

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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: 6.11 (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, 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, fenpropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydroalazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephentoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, 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, 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, sulfipyrazone, 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

some 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 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:** 12.5**OTHER SUBSTANCES****Simultaneous:** allobarbital, amobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methobarbital, methohexital, phenobarbital, 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

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Kanda, T.; Kutsuna, H.; Ohtsu, Y.; Yamaguchi, M. Synthesis of polymer-coated mixed-functional packing materials for direct analysis of drug-containing serum and plasma by high-performance liquid chromatography. *J.Chromatogr.A*, **1994**, *672*, 51–57 [column temp 40; cow; human; extracted carbamazepine, phenobarbital; also chloramphenicol, indomethacin, theophylline, trimethoprim; direct injection]

Ramachandran, S.; Underhill, S.; Jones, S.R. Measurement of lamotrigine under conditions measuring phenobarbitone, phenytoin, and carbamazepine using reversed-phase high-performance liquid chromatography at dual wavelengths. *Ther.Drug Monit.*, **1994**, *16*, 75–82 [also carbamazepine, lamotrigine, phenobarbital; serum; hexobarbital (IS); LOD 200 ng/mL; non-interfering ethosuximide, oxcarbazepine, primidone, valproic acid]

Romanyshyn, L.A.; Wichmann, J.K.; Kucharczyk, N.; Shumaker, R.C.; Ward, D.; Sofia, R.D. Simultaneous determination of felbamate, primidone, phenobarbital, carbamazepine, two carbamazepine metabolites, phenytoin, and one phenytoin metabolite in human plasma by high-performance liquid chromatography. *Ther.Drug Monit.*, **1994**, *16*, 90–99 [column temp 40–50°; LOQ 195–391 ng/mL; non-interfering clonazepam, valproic acid; simultaneous acetaminophen, aspirin, brompheniramine, caffeine, chlorpheniramine, dextromethorphan, dimethadione, ethosuximide, ethotoin, ibuprofen, iministilbene, mephenytoin, mephobarbital, metharbital, methsuximide, paramethadione, phenacemide, phensuximide, phenylpropanolamine, theophylline, trimethadione; extracted metabolites, carbamazepine, felbamate, phenobarbital, primidone]

Smigol, V.; Svec, F.; Fréchet, J.M.J. Novel uniformly sized polymeric stationary phase with hydrophilized large pores for direct injection HPLC determination of drugs in biological fluids. *J.Liq.Chromatogr.*, **1994**, *17*, 891–911 [direct injection; cow; plasma; also aspirin, caffeine, carbamazepine, lidocaine, salicylic acid, theobromine, theophylline]

Sudo, Y.; Akiba, M.; Sakaki, T.; Takahata, Y. Glycerylalkylsilylated silica gels for direct injection analysis of drugs in serum by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1994**, *17*, 1743–1754 [extracted carbamazepine, phenobarbital]

Bahal, N.; Nahata, M.C. Determination of phenytoin and its major metabolite, 5-(p-hydroxyphenyl)-5-phenylhydantoin in urine by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1993**, *16*, 1135–1142

Herbranson, D.E.; Kriss-Danziger, P. Development and validation of a high performance liquid chromatographic (HPLC) method for the determination of phenytoin prodrug (fosphenytoin) in solutions, parenteral formulations, and active drug substance. *J.Liq.Chromatogr.*, **1993**, *16*, 1143–1161 [stability-indicating; LOD 100 ng/mL]

Soto-Otero, R.; Mendez-Alvarez, E.; Sierra-Marcuño, G. High-performance liquid chromatographic measurement of phenytoin, phenobarbital and their major metabolites in serum, brain tissue, and urine. *J.Liq.Chromatogr.*, **1988**, *11*, 3021–3040 [rat]

Vio, L.; Mamolo, M.G.; Furlan, G. Simultaneous determination of phenobarbital, phenytoin and methylphenobarbital in tablet formulations by HPLC. *Farmaco.[Prat.]*, **1988**, *43*, 157–164

Asberg, A.; Haffner, F. Analysis of serum concentration of phenobarbital, phenytoin, carbamazepine and carbamazepine 10,11-epoxide by solvent-recycled liquid chromatography. *Scand.J.Clin.Lab.Invest.*, **1987**, *47*, 389–392

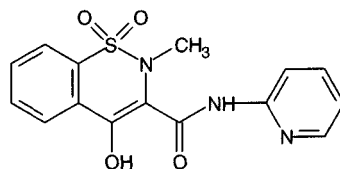
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- Neels, H.M.; Totte, J.A.; Verkerk, R.M.; Vlietinck, A.J.; Scharpe, S.L. Simultaneous high performance liquid-chromatographic determination of carbamazepine, carbamazepine-10,11-epoxide, ethosuximide, phenobarbital, phenytoin, primidone and phenylethylmalonamide in plasma. *J.Clin.Chem.Clin.Biochem.*, **1983**, *21*, 295–299
- Turnell, D.C.; Trevor, S.C.; Cooper, J.D. A rapid procedure for the simultaneous estimation of the anticonvulsant drugs, ethosuximide, phenobarbitone, phenytoin, and carbamazepine in serum using high-pressure liquid chromatography. *Ann Clin.Biochem.*, **1983**, *20 Pt 1*, 37–40
- Kinberger, B.; Holmen, A. Analysis for carbamazepine and phenytoin in serum with a high-speed liquid chromatography system (Perkin-Elmer). *Clin.Chem.*, **1982**, *28*, 718–719

Piroxicam

Molecular formula: C₁₅H₁₃N₃O₄S

Molecular weight: 331.4

CAS Registry No.: 36322-90-4, 87234-24-0 (cinnamic acid ester),
85056-47-9 (piroxicam olamine)



SAMPLE

Matrix: bile, blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 1 mL 10 mM NaOH + 100 μ L 100 μ g/mL isoxicam in 10 mM NaOH + 250 μ L 1 M HCl + 5 mL diethyl ether, shake for 6 min at 240 rpm on an orbital shaker, centrifuge at 4° at 900 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 250 μ L MeCN:water 50:50, vortex for 1 min, inject a 50 μ L aliquot. Urine, bile. 500 μ L Urine or bile + 1 mL 10 mM NaOH + 100 μ L 100 μ g/mL isoxicam in 10 mM NaOH + 250 μ L 1 M HCl + 5 mL diethyl ether, shake for 6 min at 240 rpm on an orbital shaker, centrifuge at 4° at 900 g for 10 min. Remove the organic layer and add it to 2 mL pH 4.9 citric acid-phosphate buffer, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 250 μ L MeCN:water 50:50, vortex for 1 min, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: Techsil C10 CN (HPLC Technology)

Column: 200 \times 3.9 10 μ m Techsil C10 CN (HPLC Technology)

Mobile phase: MeCN:water 22:78 (plasma, urine) or 10:90 (bile) containing 50 mM NaH₂PO₄, pH 3.5

Flow rate: 2.5

Injection volume: 50

Detector: UV 365

CHROMATOGRAM

Retention time: 4 (plasma), 6.8 (bile)

Internal standard: isoxicam (8 (plasma), 17 (bile))

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; do not use plasticware

REFERENCE

Milligan, P.A. Determination of piroxicam and its major metabolites in the plasma, urine and bile of humans by high-performance liquid chromatography. *J.Chromatogr.*, **1992**, *576*, 121–128

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 20 μ L ketoprofen solution, mix, add 500 μ L MeCN, vortex for 10 s, centrifuge at 1500 g for 10 min. Remove 800 μ L of the supernatant and add it to 5 mL dichloromethane, vortex for 30 s, centrifuge for 10 min, inject a 50 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 20 \times 4.6 10 μ m Spherisorb C8

Column: 250 × 4.6 5 µm Spherisorb ODS

Mobile phase: MeOH:buffer 40:60 (Buffer was 40 mM Na₂HPO₄ adjusted to pH 8 with orthophosphoric acid.)

Column temperature: 50

Flow rate: 1

Injection volume: 50

Detector: UV 360

CHROMATOGRAM

Retention time: 4.4

Internal standard: ketoprofen (8.6)

Limit of detection: 100 ng/mL

Limit of quantitation: 300 ng/mL

KEY WORDS

plasma

REFERENCE

Edno, L.; Bressolle, F.; Combe, B.; Galtier, M. A reproducible and rapid HPLC assay for quantitation of piroxicam in plasma. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 785–789

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 µL 20 µg/mL isoxicam in MeOH + 200 µL 1 M HCl, vortex at slow speed for 30 s, add 10 mL dichloromethane, shake vigorously for 30 s, centrifuge at 2500 rpm for 5 min. Remove the organic phase and add it to 20 mg anhydrous sodium sulfate, filter, evaporate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 µL MeOH, vortex for 30 s, centrifuge at 15000 g for 5 min, inject 20 µL of the supernatant.

HPLC VARIABLES

Column: 250 × 4 5 µm LiChrospher 60 RP-Select B

Mobile phase: MeOH:water:acetic acid 48:45:7, pH 2.47

Flow rate: 1.1

Injection volume: 20

Detector: UV 340

CHROMATOGRAM

Retention time: 6.1

Internal standard: isoxicam (9.75)

Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: droxicam

KEY WORDS

protect from light; plasma; pharmacokinetics

REFERENCE

Maya, M.T.; Pais, J.P.; Morais, J.A. A rapid method for the determination of piroxicam in plasma by high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 319–322

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 μ m 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 338

CHROMATOGRAM

Retention time: 4.20

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, albuterol, alimemazine, alminoprofen, alpidem, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benzazepil, benperidol, benzocaine, benzoylegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, clobazepam, cicletanine, clemastine, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cyproheptadine, cytarabine, dacarbazine, daunorubicin, demexiptiline, desipramine, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mepredine, mephenesin, mepivacaine, metapramine, metformin, methadone, methocarbamol, methotrexate, metipranolol, metoclopramide, mexiletine, mianserine, midazolam, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, niflumic acid, nimodipine, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxprenolol, penbutolol, penfluridol, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, prazepam, prazosin, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinupramine, ramipril, ranitidine, reserpine, ritrodine, secobarbital, sotalol, strychnine, sulfipyrazole, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thio-properazine, thioridazine, tianeptine, tiapride, ticlopidine, timolol, tiocloamarol, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, zolpidem, zorubicine

Interfering: ajmaline, alprazolam, celiprolol, clobazam, cycloguanil, debrisoquine, dextromethorphan, disopyramide, ketamine, lorazepam, mephentermine, methaqualone, metoprolol, minoxidil, nifedipine, nitrazepam, oxazepam, pentazocine, prilocaine, quinidine, quinine, sulindac, tiaprofenic acid, tofisopam, yohimbine, zopiclone

KEY WORDS

whole blood; plasma

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: Inject 20 μL plasma onto column A with mobile phase A, after 1 min switch mobile phase A to drain and leave column A to sit for 1 min. Elute the contents of column A onto column B with mobile phase B. After 1 min remove column A from circuit and re-equilibrate it with mobile phase A. Elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES

Column: A 100 \times 2 40 μm Bond Elut C2 (Chrompack) (Before use condition with MeCN, water, mobile phase A. Replace column after 40 injections.); B 20 \times 4.6 Supelcosil LC18 DB + 150 \times 4.6 5 μm Supelcosil LC18 DB

Mobile phase: A 150 mM NaH_2PO_4 adjusted to pH 3.5 with orthophosphoric acid; B MeCN:20 mM Na_2HPO_4 + 0.5 mM triethylamine adjusted to pH 3.1 with orthophosphoric acid 60:40

Flow rate: A 1; B 1

Injection volume: 20

Detector: UV 331

CHROMATOGRAM

Retention time: 5.25

Limit of detection: 100 ng/mL

Limit of quantitation: 200 ng/mL

KEY WORDS

plasma; column-switching

REFERENCE

Saeed, K.; Becher, M. On-line solid-phase extraction of piroxicam prior to its determination by high-performance liquid chromatography. *J. Chromatogr.*, **1991**, *567*, 185–193

SAMPLE

Matrix: blood

Sample preparation: Animal. 100 μL Plasma + 100 μL 1 M HCl + 100 μL 20 $\mu\text{g/mL}$ naproxen in 2% MeOH + 6 mL MTBE, vortex for 1 min, centrifuge at 1200 g for 2 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35°. Reconstitute in 1 mL 2% MeOH, vortex for 2 min, inject a 100 μL aliquot. Human. 500 μL Plasma + 200 μL 1 M HCl + 100 μL 100 $\mu\text{g/mL}$ naproxen in 2% MeOH + 8 mL diethyl ether, vortex for 1 min, centrifuge at 1200 g for 2 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35°. Reconstitute in 2 mL 2% MeOH, vortex for 2 min, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 50 \times 4.6 5 μm Spherisorb C8

Mobile phase: MeCN:THF:MeOH:2% acetic acid (pH 2.5) 5:18:5:72

Flow rate: 1.5

Injection volume: 100

Detector: UV 330 (animal); UV 360 (human)

CHROMATOGRAM**Retention time:** 2.2**Internal standard:** naproxen (8) (determined at UV 330 nm in each case)**Limit of quantitation:** 250 ng/mL

OTHER SUBSTANCES**Extracted:** metabolites, 5'-hydroxypiroxicam

KEY WORDSplasma; dog; rat; human

REFERENCEGillilan, R.B.; Mason, W.D.; Fu, C.-H.J. Rapid analysis of piroxicam in dog, rat and human plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1989**, *487*, 232–235

SAMPLE**Matrix:** blood**Sample preparation:** 50 μ L Serum + 20 μ L 100 mM pH 4.8 citrate buffer + 5 μ L 30 μ g/mL isoxicam in MeOH + 3 mL dichloromethane, shake for 45 s, centrifuge at 4000 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μ L mobile phase, mix for 10 s, centrifuge, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 7 μ m Separon SGX CN**Mobile phase:** MeCN:10 mM phosphoric acid 70:30**Flow rate:** 0.4**Injection volume:** 20**Detector:** UV 350

CHROMATOGRAM**Retention time:** 4.10**Internal standard:** isoxicam (2.74)**Limit of detection:** 40 ng/mL

KEY WORDSserum

REFERENCEMigulla, H.; Alken, R.G.; Hüller, H. Mikromethode zur Bestimmung der Piroxicamkonzentration im Serum [Micromethod for the determination of piroxicam concentration in serum]. *Pharmazie*, **1988**, *43*, 866–867

SAMPLE**Matrix:** blood, exudate**Sample preparation:** 150 μ L Plasma or exudate + 50 μ L 5 μ g/mL 6-methylpiroxicam + 200 μ L 1 M pH 2 phosphate buffer, vortex, add 2 mL dichloromethane, rotate for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 120 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES**Column:** 200 \times 4.6 Hypersil ODS**Mobile phase:** MeOH:pH 8 phosphate buffer 45:55**Flow rate:** 1

Injection volume: 100

Detector: UV 360

CHROMATOGRAM

Internal standard: 6-methylpiroxicam

Limit of detection: 10 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, tissue

Sample preparation: 1 mL Plasma or tissue + 50 μ L 100 μ g/mL isoxicam in MeOH + 700 mg potassium carbonate + 1 mL THF + 500 μ L EtOH, 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 residue in 100 μ L THF, vortex for 5 s, filter (0.5 μ m), inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 μ m Novapak C18

Mobile phase: THF:water 45:55 with 1% acetic acid and 5 mM 1-heptanesulfonic acid (PIC B-7, Waters)

Flow rate: 0.7

Injection volume: 20

Detector: UV 313

CHROMATOGRAM

Retention time: 4.2

Internal standard: isoxicam (8.0)

Limit of detection: 100 ng/mL

KEY WORDS

plasma; rat; skin; muscle

REFERENCE

Carretani, D.; Micheli, L.; Fiaschi, A.I.; Giorgi, G. Rapid and sensitive determination of piroxicam in rat plasma, muscle and skin by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *614*, 103-108

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μ L Plasma + 200 μ L 10% trichloroacetic acid + 100 μ L 15 μ g/mL naproxen in MeOH + 700 μ L MeOH, vortex for 30 s, centrifuge at 2105 g for 10 min, inject a 100 μ L aliquot of the supernatant. Urine. 1 mL Urine + 400 μ L 1 M NaOH, vortex, let stand at room temperature for 2 h, add 300 μ L MeOH, add 100 μ L 15 μ g/mL naproxen in MeOH, mix, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: 100 \times 2 Spherisorb pellicular reversed-phase

Column: 250 \times 4.5 μ m Spherisorb C18

Mobile phase: MeCN:100 mM sodium acetate 33:67, adjusted to pH 3.3 with glacial acetic acid

Column temperature: 40

Flow rate: 2.5

Injection volume: 100

Detector: UV 330

CHROMATOGRAM

Retention time: 2.6

Internal standard: naproxen (3.2)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Avgerinos, A.; Axarlis, S.; Dragatsis, J.; Karidas, T.; Malamataris, S. Extractionless high-performance liquid chromatographic method for the simultaneous determination of piroxicam and 5'-hydroxy-piroxicam in human plasma and urine. *J.Chromatogr.B*, **1995**, 673, 142-146

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μ L Plasma or urine + 1 mL 100 mM NaOH + 100 μ L 100 μ g/mL isoxicam in 10 mM NaOH + 250 μ L 1 M HCl + 5 mL diethyl ether, shake mechanically for 5 min, centrifuge at 1150 g at 4° for 5 min. Remove the ether layer and evaporate it to dryness at 35° under a stream of nitrogen. Reconstitute the residue in 250 μ L 50 mM TRIS, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 10 μ m μ Bondapak CN (plasma) or 300 \times 3.9 10 μ m μ Bondapak C18 (urine)

Mobile phase: MeCN:water 25:75 containing 50 mM NaH_2PO_4 , final pH 3.2 (plasma) or THF:5 mM sodium octylsulfonate buffer:glacial acetic acid 45:54:1 (urine)

Flow rate: 1.5

Injection volume: 50

Detector: UV 365

CHROMATOGRAM

Retention time: 3 (plasma), 4 (urine)

Internal standard: isoxicam (6 (plasma), 7 (urine))

Limit of detection: 50 ng/mL (plasma); 100 ng/mL (urine)

OTHER SUBSTANCES

Extracted: 5'-hydroxy-piroxicam

KEY WORDS

plasma

REFERENCE

Richardson, C.J.; Ross, S.G.; Blocka, K.L.; Verbeeck, R.K. High-performance liquid chromatographic analysis of piroxicam and its major metabolite 5'-hydroxy-piroxicam in human plasma and urine. *J.Chromatogr.*, **1986**, 382, 382-388

SAMPLE**Matrix:** feed, formulations**Sample preparation:** Capsules. Weigh out contents of a capsule (5-20 mg piroxicam), shake with 70 mL 10 mM HCl in MeOH for 30 min, make up to 100 mL with 10 mM HCl in MeOH, centrifuge at 2000 rpm for 10 min, dilute to 50 µg/mL with 10 mM HCl in MeOH before analysis, inject a 25 µL aliquot, use UV determination at 254 nm. Tablets (Formulation I). Disintegrate tablet with 5 mL water, shake with 70 mL 10 mM HCl in MeOH for 30 min, make up to 100 mL with 10 mM HCl in MeOH, centrifuge at 2000 rpm for 10 min, dilute to 50 µg/mL with 10 mM HCl in MeOH before analysis, inject a 25 µL aliquot, use UV determination at 254 nm. Tablets (Formulation II). Shake with 70 mL 10 mM NaOH in MeOH for 30 min, make up to 100 mL with MeOH:water 45:55, centrifuge at 2000 rpm for 10 min, dilute to 50 µg/mL with MeOH:water 45:55 before analysis, inject a 25 µL aliquot, use UV determination at 254 nm. Ointment. Dissolve 500 mg 10% ointment in 4 mL chloroform, warm if necessary, make up to 100 mL with 10 mM HCl in MeOH, mix, filter (Whatman GF/A), discard first few mL of filtrate, dilute to tenfold with 10 mM HCl in MeOH before analysis, inject a 25 µL aliquot, use UV detection at 340 nm. Suppositories. Weigh out, dissolve completely in 20 mL warm MeOH:isopropanol 1:1, add 60 mL mobile phase, shake for 20 min, make up to 100 mL with mobile phase, mix, filter (Whatman GF/A), dilute if necessary with mobile phase, inject a 25 µL aliquot, use UV determination at 254 nm. Ophthalmic suspensions. Shake until homogeneous, dilute a 5 mL aliquot to 100 mL with MeOH, dilute 10 fold with mobile phase, adjust pH to 4.0, inject a 20 µL aliquot, adjust mobile phase pH to 4.0. Rodent feed. Shake a 10 g sample with 25 mL MeCN:glacial acetic acid 90:10 for 30 min (or up to 6 h for aged samples), centrifuge at 1500 rpm for 10 min, inject a 50 µL aliquot, use UV determination at 365 nm, adjust mobile phase pH to 4.0.

HPLC VARIABLES**Column:** 300 × 3.9 10 µm Waters ODS, cat no. 27324**Mobile phase:** MeOH:buffer 45:55 (Buffer was 400 mL 100 mM citric acid, add 200 mM Na₂HPO₄·7H₂O until pH = 3 (about 102 mL), dilute to 1 L with water.)**Flow rate:** 1.2**Injection volume:** 25-50**Detector:** UV 254; UV 340; UV 365

CHROMATOGRAM**Retention time:** 10

OTHER SUBSTANCES**Also analyzed:** impurities, degradation products, metabolites, piroxicam analogues

KEY WORDS

capsules; tablets; ointments; suppositories; ophthalmic suspensions; stability-indicating

REFERENCERichards, J.A.; Cole, D.A.; Hickey, R.J.; Sokol, L.S.-W. High performance liquid chromatography assay for piroxicam in pharmaceutical products. *J.Chromatogr.Sci.*, 1987, 25, 292-295

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 125 × 3 Ecocart LiChrospher 100 RP-18**Mobile phase:** Isopropanol:100 mM KH₂PO₄:formic acid 54:100:0.1**Flow rate:** 0.6**Detector:** UV 254

CHROMATOGRAM**Retention time:** 2.3**Limit of quantitation:** 200-500 ng/mL

OTHER SUBSTANCES**Simultaneous:** acemetacin, diclofenac, flurbiprofen, indomethacin, ketoprofen, lonazolac, naproxen, sulindac, tenoxicam

REFERENCEBaeyens, 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:** 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:** 6.84 (A), 6.67 (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, cycizine, 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, methyl dopa, 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, 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, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline,

thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinamide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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

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- Bartlett, A.; Costa, A.; Martínez, L.; Roser, R.; Sagarra, R.; Sánchez, J. The effect of antacid and ranitidine on droxicam pharmacokinetics. *J.Clin.Pharmacol.*, **1992**, 32, 1115–1119 [plasma; isoxicam (IS); LOD 25 ng/mL]
- Jiang, X.G.; Ge, S.D.; Wang, X.J.; Xi, N.Z. [A HPLC method for determining piroxicam in body fluids]. *Chung Kuo Yao Li Hsueh Pao*, **1991**, 12, 381–384
- Wanwimolruk, S.; Wanwimolruk, S.Z.; Zoest, A.R. A simple and sensitive HPLC assay for piroxicam in plasma and its application to bioavailability study. *J.Liq.Chromatogr.*, **1991**, 14, 2373–2381 [isoxicam (IS); LOD 50 ng/mL; microbore]
- Sánchez, J.; Martínez, L.; García-Barbal, J.; Roser, R.; Bartlett, A.; Sagarra, R. The influence of gastric emptying on droxicam pharmacokinetics. *J.Clin.Pharmacol.*, **1989**, 29, 739–745 [plasma; LOD 100 ng/mL]
- Boudinot, F.D.; Ibrahim, S.S. High-performance liquid chromatographic assay for piroxicam in human plasma. *J.Chromatogr.*, **1988**, 430, 424–428 [6'-methylpiroxicam (IS); LOD 2 ng/mL; pharmacokinetics]
- Ge, S.D.; Cheng, Q.Y.; Wang, X.J.; Xi, N.Z. [HPLC determination of piroxicam contents in piroxicam suppositories]. *Yao Hsueh Hsueh Pao*, **1988**, 23, 38–41
- Ahmed, A.N.; El-Gizawy, S.M. Use of chemically-bonded cyclodextrin stationary phase for high performance liquid chromatographic determination of Feldene capsules. *J.Chromatogr.Sci.*, **1987**, 25, 424–426 [column temp 35]
- Macek, J.; Vacha, J. Rapid and sensitive method for determination of piroxicam in human plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, 420, 445–449 [column temp 35; glass column; LOD 150 ng/mL; pharmacokinetics; simultaneous antipyrine, diclofenac, hydroxychloroquine, ibuprofen, isoxicam, plaquenil, sulfamethazine]
- Chen, X.L.; Xi, N.Z.; Ge, S.D.; Sun, S.L. [HPLC determination of piroxicam in human serum and its pharmacokinetic parameters]. *Yao Hsueh Hsueh Pao*, **1986**, 21, 692–697
- Dixon, J.S.; Lowe, J.R.; Galloway, D.B. Rapid method for the determination of either piroxicam or tenoxicam in plasma using high-performance liquid chromatography. *J.Chromatogr.*, **1984**, 310, 455–459
- Fraser, A.D.; Woodbury, J.F. Liquid chromatographic determination of piroxicam in serum. *Ther.Drug Monit.*, **1983**, 5, 239–242
- Twomey, T.M.; Bartolucci, S.R.; Hobbs, D.C. Analysis of piroxicam in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1980**, 183, 104–108

Polymyxin

Molecular formula: C₅₆H₉₈N₁₆O₁₃ (B₁)

Molecular weight: 1203.5 (B₁)

CAS Registry No.: 1406-11-7 (polymyxin), 1404-26-8 (polymyxin B), 1405-20-5 (polymyxin B sulfate)

SAMPLE

Matrix: formulations

Sample preparation: Sandwich cream or ointment between two layers of 200 mesh silica gel, extract with carbon dioxide:MeOH 95:5 at 300 atmospheres at 55° at 2 mL/min for 75 min (restrictor 300°), sonicate the SFE tube, frits, and silica gel with MeOH:100 mM HCl 25:75 containing 0.1% Tween 80 for 15 min, filter (0.2 μm), inject an aliquot of the filtrate. (SFE removes the hydrocarbon base of the cream or ointment leaving behind the insoluble polymyxin.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Synchronapak SCD

Mobile phase: MeCN:buffer 21.5:78.5 (Buffer was 100 mM KH₂PO₄ containing 0.1% trifluoroacetic acid.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 215

CHROMATOGRAM

Limit of quantitation: 0.016%

KEY WORDS

SFE; cream; ointment

REFERENCE

Moore, W.N.; Taylor, L.T. Analytical inverse supercritical fluid extraction of polar pharmaceutical compounds from cream and ointment matrices. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1227–1232

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 45 × 4.7 Ultrasphere C18

Column: 250 × 4.7 Ultrasphere C18

Mobile phase: Gradient. A was 0.15% trifluoroacetic acid in water. B was 0.15% trifluoroacetic acid in MeCN. A:B from 100:0 to 50:50 over 25 min.

Flow rate: 2

Detector: UV 215

CHROMATOGRAM

Retention time: 18.5

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Danner, R.L.; Joiner, K.A.; Rubin, M.; Patterson, W.H.; Johnson, N.; Ayers, K.M.; Parrillo, J.E. Purification, toxicity, and antiendotoxin activity of polymyxin B nonapeptide. *Antimicrob.Agents Chemother.*, **1989**, *33*, 1428–1434

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 10 μ L aliquot of a 1 mg/mL solution.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Vydac TP C18**Mobile phase:** Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.075% trifluoroacetic acid in MeCN. A:B from 90:10 to 20:80 over 20 min.**Flow rate:** 1.2**Injection volume:** 10**Detector:** UV 215

CHROMATOGRAM**Retention time:** 12.0

OTHER SUBSTANCES**Simultaneous:** degradation products.

REFERENCEVaara, M. Analytical and preparative high-performance liquid chromatography of the papain-cleaved derivative of polymyxin B. *J.Chromatogr.*, **1988**, *441*, 423–430

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 400-500 μ g/mL solution in water, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Nucleosil 5 C18**Mobile phase:** MeCN:buffer 23:77 (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 (polymyxin B2), 15 (polymyxin B1)

OTHER SUBSTANCES**Also analyzed:** colistin (polymyxin E)

REFERENCEElverdam, 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 μ g/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Ultrasphere ion-pair**Mobile phase:** MeCN:buffer 23:77 (Prepare mobile phase by mixing 230 mL MeCN, 700 mL water, and 38 g Na₃PO₄, adjust pH to 3.0 with phosphoric acid, make up to 1 L with water.)**Column temperature:** 27**Flow rate:** 1

Injection volume: 10
Detector: UV 185; UV 200

CHROMATOGRAM

Retention time: 7 (B₂), 8 (B₃), 13 (B₁)
Limit of detection: 30 ng (UV 185)

OTHER SUBSTANCES

Simultaneous: colistin

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: Inject a 5-50 μ L aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES

Column: 200 \times 4 Nucleosil 5C18
Mobile phase: MeCN:buffer 22.5:77.5 (Buffer was 5 mM pH 3.0 tartrate buffer containing 5 mM sodium 1-butanefulfonate and 50 mM sodium sulfate.)
Flow rate: 1
Injection volume: 5-50
Detector: UV 220

CHROMATOGRAM

Retention time: 11 (polymyxin B₂), 13 (polymyxin B₃), 23.5 (polymyxin B₁)

OTHER SUBSTANCES

Simultaneous: colistin, polymyxin C, polymyxin D, polymyxin E, polymyxin M, polymyxin S, polymyxin T

REFERENCE

Terabe, S.; Konaka, R.; Shoji, J. Separation of polymyxins and octapeptins by high-performance liquid chromatography. *J.Chromatogr.*, **1979**, *173*, 313–320

ANNOTATED BIBLIOGRAPHY

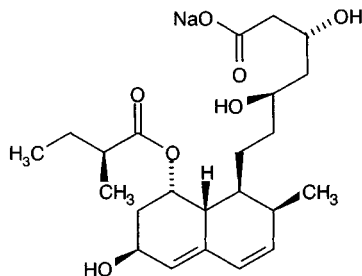
Kitamura-Matsunaga, H.; Kimura, Y.; Araki, T.; Izumiya, N. A sodium-containing polymyxin derived from polymyxin-complex during chromatography. *J.Antibiot.(Tokyo)*, **1984**, *37*, 1605–1610 [for colistin, polymyxin E; analytical; preparative]

Pravastatin

Molecular formula: C₂₃H₃₆O₇

Molecular weight: 423.5

CAS Registry No.: 81131-70-6 (pravastatin sodium),
81093-37-0 (pravastatin)



SAMPLE

Matrix: blood

Sample preparation: Condition an immobilized antibody column (preparation details in paper) with 4 mL water, 4 mL MeOH:water 80:20, and 4 mL PBS. Add 1 mL plasma to the column, let stand for 15 min, wash with two 4 mL portions of water, wash with 4 mL PBS, wash with two 4 mL portions of water, elute with 4 mL MeOH. Add 100 μ L 100 ng/mL IS in water to the eluate, evaporate to dryness, reconstitute with 100 μ L DMF, add 100 μ L 47.6 μ g/mL triethylamine in dioxane (Caution! Dioxane is a carcinogen!), add 100 μ L 51.8 μ L/mL diethyl phosphorocyanidate in dioxane, add 100 μ L 100 μ g/mL N-dansylethylenediamine (Molecular Probes, Inc., Eugene OR) in dioxane, stir for 10 s, let stand at room temperature for 10 min, inject a 10 μ L aliquot onto column A and elute to waste with mobile phase A. After 7.5 min elute the contents of column A onto column B with mobile phase B. After 2.5 min remove column A from the circuit. Elute column B with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES

Column: A 150 \times 4.6 Ultron 300-C4 (Shinwa Chemical Industries); B 150 \times 6 Cosmosil 5C18-AR (Nacalai Tesque)

Mobile phase: A MeCN:10 mM pH 2.4 citric acid 30:70; B MeCN:5 mM pH 2.6 citric acid 50:50

Flow rate: 1

Injection volume: 10

Detector: F ex 325 (40 mW He-Cd laser); F ex 350 em 530; UV 239

CHROMATOGRAM

Retention time: 17.3

Internal standard: R-416 (Sankyo) (19)

Limit of quantitation: 0.1 ng/mL (laser fluorescence)

KEY WORDS

column-switching; dog; rat; laser; plasma; SPE; derivatization; pharmacokinetics

REFERENCE

Dumousseaux, C.; Muramatsu, S.; Takasaki, W.; Takahagi, H. Highly sensitive and specific determination of pravastatin sodium in plasma by high-performance liquid chromatography with laser-induced fluorescence detection after immobilized antibody extraction. *J.Pharm.Sci.*, **1994**, *83*, 1630-1636

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL C2 Bond-Elut SPE cartridge with 2 mL water, 2 mL MeOH, and 2 mL water. 1 mL Plasma + 1 mL 100 mM pH 7.2 KH₂PO₄, add to the SPE cartridge, wash with 2 mL water, elute with 500 μ L MeCN:water 75:25. Evaporate the eluate to dryness under reduced pressure, reconstitute with 80 μ L IS solution, cen-

trifuge, inject a 50 μL aliquot. (Prepare the IS solution by mixing 2 mL 100 $\mu\text{g}/\text{mL}$ simvastatin β -hydroxyacid in MeOH, 1 mL MeOH, and 1 mL 100 mM pH 5 KH_2PO_4 .)

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18

Column: 50 \times 4.6 3 μm Supelcosil LC-18

Mobile phase: MeCN:50 mM pH 3.5 ammonium phosphate 26:74

Column temperature: 50

Flow rate: 1.6

Injection volume: 50

Detector: UV 238

CHROMATOGRAM

Retention time: 3.0

Internal standard: simvastatin β -hydroxyacid (4.8)

Limit of detection: 2 ng/mL

KEY WORDS

pharmacokinetics; plasma; SPE

REFERENCE

Iacona, I.; Regazzi, M.B.; Buggia, I.; Villani, P.; Fiorito, V.; Molinaro, M.; Guarnone, E. High-performance liquid chromatography determination of pravastatin in plasma. *Ther. Drug Monit.*, **1994**, *16*, 191–195

SAMPLE

Matrix: blood, feces, urine

Sample preparation: Plasma. 2 mL Plasma + 4 mL MeCN, centrifuge at 700 g for 10 min, remove the supernatant and wash the precipitate twice with 2 mL MeCN:water 2:1. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute the residue in 1 mL MeCN:water 2:1, centrifuge at 10000 g, remove the supernatant and add it to 500 μL water, centrifuge at 10000 g, inject a 1 mL aliquot. Urine. Centrifuge at 10000 g, inject an aliquot. Feces. 1 g Homogenized feces + 2 mL MeCN, sonicate for 5 min, shake in a wrist action shaker for 20 min, centrifuge at 700 g for 10 min. Remove the supernatant and wash the precipitate twice with 1 mL MeCN:water 2:1, combine the supernatants, inject a 500 μL aliquot.

HPLC VARIABLES

Guard column: present but not specified

Column: 500 \times 9.4 Partisil 10 ODS-3 C18

Mobile phase: Gradient. MeCN:10 mM pH 7.2 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulphate at 25:75 for 20 min, then to 50:50 over 45 min, hold at 50:50 for 10 min.

Flow rate: 4

Injection volume: 500-1000

Detector: Collect fractions and measure radioactivity; UV 245

CHROMATOGRAM

Retention time: 33

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; semi-preparative; radiolabeled starting material

REFERENCE

Everett, D.W.; Chando, T.J.; Didonato, G.C.; Singhvi, S.M.; Pan, H.Y.; Weinstein, S.H. Biotransformation of pravastatin sodium in humans. *Drug Metab.Dispos.*, **1991**, *19*, 740–748

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES

Column: Cosmosil 5C18-AR (Nacalai Tesque)

Mobile phase: A MeCN:10 mM pH 2.4 citric acid 30:70

Flow rate: 1

Detector: UV 235

REFERENCE

Dumousseaux, C.; Muramatsu, S.; Takasaki, W.; Takahagi, H. Highly sensitive and specific determination of pravastatin sodium in plasma by high-performance liquid chromatography with laser-induced fluorescence detection after immobilized antibody extraction. *J.Pharm.Sci.*, **1994**, *83*, 1630–1636

SAMPLE

Matrix: solutions

Sample preparation: Centrifuge at 2000 rpm, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak

Mobile phase: MeOH:water:triethylamine:glacial acetic acid 500:500:1:1

Column temperature: 30

Flow rate: 1.3

Detector: UV 238

CHROMATOGRAM

Retention time: 17.5 (10.1 hydroxy acid form)

Limit of detection: 10 ng/mL

Limit of quantitation: 25 ng/mL

REFERENCE

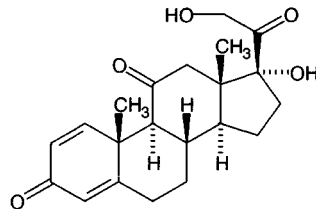
Serajuddin, A.T.; Ranadive, S.A.; Mahoney, E.M. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin. *J.Pharm.Sci.*, **1991**, *80*, 830–834

Prednisone

Molecular formula: C₂₁H₂₆O₅

Molecular weight: 358.4

CAS Registry No.: 53-03-2



SAMPLE

Matrix: blood

Sample preparation: Condition an Empore C8 SPE extraction disc (3M Co.) by adding 500 μ L MeOH and forcing through three drops, discard the remaining liquid, add water, force through three drops, discard the water. 300 μ L Serum + 150 μ L IS solution, let stand at room temperature for 10 min, add 800 μ L saturated sodium borate solution, mix, centrifuge at 12400 g for 3 min (if necessary), add to SPE extraction disc, centrifuge at 100-120 g for 5 min, force through 200 μ L water, force through 500 μ L MeOH:water 18:82, elute with 50 μ L MeCN then 150 μ L water, mix the eluates, inject a 20 μ L 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 \times 2 30 μ m Permaphase ETH (Du Pont)

Column: 250 \times 2 Ultrasphere C18 or 250 \times 4.6 Ultrasphere C18

Mobile phase: THF:water 20:80 (Use a 150 \times 4.6 37-53 μ m silica gel (Whatman) saturating column (held at 55°) between the pump and the injector.)

Column temperature: 55

Flow rate: 0.18 (250 \times 2) or 0.8 (250 \times 4.6)

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 10

Internal standard: fludrocortisone (15), methylprednisolone (20)

Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, cortisone, hydrocortisone, prednisolone

Simultaneous: aldosterone, androsteindione, beclomethasone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisone, dexamethasone, 17-hydroxyprogesterone, metyrapone, pregnenolone, progesterone, testosterone, triamcinolone

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 μ L water containing 5 μ g/mL 2,3-diaminonaphthalene and 3.5 μ g/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 μ L MeOH:100 mM perchloric acid 50:50, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m Nova-Pak C18

Mobile phase: Gradient. A was 58 mM NaH₂PO₄ 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 83:17 over 5 min, to 75:25 over 12 min, to 70:30 over 4 min, to 65:35 over 9 min.

Flow rate: 1

Injection volume: 20

Detector: UV 245; UV 256; UV 343

CHROMATOGRAM

Retention time: 14.15

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, dexamethasone, fluen-drenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17 δ -hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, progesterone, triamcinolone

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE

Volin, P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J. Chromatogr. B*, **1995**, 666, 347-353

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 5 mL MeOH, vortex for 10 s, centrifuge at 2000 g for 10 min, inject a 15 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: CN Guard-Pak

Column: 150 \times 4.6 5 μ m APEX octyl EC C8 (Jones Chromatography)

Mobile phase: Gradient. MeOH:buffer from 55:45 to 20:80 over 20 min. (Buffer was water acidified to pH 3.0 with trifluoroacetic acid.)

Flow rate: 1.5

Injection volume: 15

Detector: UV 254

CHROMATOGRAM

Retention time: 5.5

Internal standard: prednisone

OTHER SUBSTANCES

Extracted: novobiocin

KEY WORDS

plasma; prednisone is IS

REFERENCE

Chen, T.-L.; Kennedy, M.J.; Dunlap, V.M.; Colvin, O.M. Determination of plasma novobiocin levels by a reversed-phase high-performance liquid chromatographic assay. *J.Chromatogr.B*, **1994**, *652*, 109–113

SAMPLE

Matrix: blood

Sample preparation: Prepare a Sep-Pak Plus Environmental C18 SPE cartridge by washing with 15 mL MeOH then 15 mL water. 1 mL Serum + 100 μ L 3 μ g/mL betamethasone in isopropanol:MeCN 1:1 + 100 μ L isopropanol:acetonitrile 1:1, mix, add to the 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 μ L mobile phase A, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 guard column

Column: 250 \times 4.6 5 μ m 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: 23

Internal standard: betamethasone (33)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, cortisone, hydrocortisone, prednisolone

KEY WORDS

serum; SPE

REFERENCE

Hirata, H.; Kasama, T.; Sawai, Y.; Fike, R.R. Simultaneous determination of deflazacort metabolites II and III, 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: 1 mL Plasma + 50 μ L 4 μ g/mL betamethasone in EtOH + 15 mL dichloromethane, shake horizontally for 15 min, centrifuge at 1500 g for 15 min. Remove the organic layer and wash it with 100 μ L 100 mM NaOH then 1 mL water. Remove the aqueous phase and dry the organic phase over 1 g of anhydrous sodium sulfate. Evaporate the organic phase to dryness under a stream of nitrogen at not more than 37°, reconstitute in 200 μ L mobile phase, inject a 175 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 2 30-38 μ m HC Pellosil

Column: 250 \times 4.6 5-6 μ m Zorbax SIL

Mobile phase: Heptane:dichloromethane:glacial acetic acid:ethanol 350:600:10:35

Flow rate: 2

Injection volume: 175

Detector: UV 254

CHROMATOGRAM

Retention time: 9

Internal standard: betamethasone (12)

Limit of detection: 5 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: hydrocortisone, prednisolone

Simultaneous: hydrocortisone, prednisolone

Noninterfering: cyclosporin, ethinyl estradiol, ketoconazole, levonorgestrel, rapamycin, tacrolimus, tenidap, tetrahydrocortisone

KEY WORDS

plasma; normal phase

REFERENCE

Jusko, W.J.; Pyszczynski, N.A.; Bushway, M.S.; D'Ambrosio, R.; Mis, S.M. Fifteen years of operation of a high-performance liquid chromatographic assay for prednisolone, cortisol and prednisone in plasma. *J.Chromatogr.B*, **1994**, 658, 47-54

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 2 μ g/mL 11-deoxy-17-hydroxycorticosterone in MeOH, mix for 5 s, add 7 mL dichloromethane, shake on a mechanical shaker for 5 min. Remove the organic phase and add it to 2 mL 100 mM HCl, shake, centrifuge for 5 min, wash the organic layer with 2 mL 200 mM NaOH, wash the organic layer with 2 mL water. Evaporate the organic layer to dryness, reconstitute the residue with 75 μ L mobile phase, mix for 20 s, centrifuge at 10000 g for 2 min, inject 30 μ L of the supernatant

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb C6

Mobile phase: MeOH:water 40:60

Flow rate: 1.4

Injection volume: 30

Detector: UV 254

CHROMATOGRAM

Retention time: 11.8

Internal standard: 11-deoxy-17-hydroxycorticosterone (29.4)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: prednisolone

Simultaneous: acetaminophen, aldosterone, allopurinol, amitriptyline, caffeine, calcitriol, cephalothin, chlordiazepoxide, chlorothiazide, corticosterone, cortisone, dexamethasone, diazepam, ephedrine, ethinyl estradiol, furosemide, hydrocortisone, ibuprofen, imipramine, indomethacin, mechllorethamine, methylprednisone, metolazone, nandrolone, naproxen, phenacetin, phenobarbital, phenytoin, probenecid, progesterone, propranolol, sulfasalazine, testosterone, theophylline, vincristine

KEY WORDS

plasma

REFERENCE

Cheng, M.H.; Huang, W.Y.; Lipsey, A.I. Simultaneous liquid-chromatographic determination of prednisone and prednisolone in plasma. *Clin.Chem.*, **1988**, *34*, 1897-1899

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 40 μ L 5 μ g/mL dexamethasone in MeOH, vortex 30 s, add 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 μ L dichloromethane, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 17

Internal standard: dexamethasone (23.5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: cortisone, hydrocortisone, prednisolone, prednisolone acetate

KEY WORDS

plasma; normal phase; pig

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 Bond-Elut C18 SPE cartridge with 2 mL MeCN, 2 mL acetone:water 2:98, and 4 mL water. Do not allow cartridge to run dry. 2 mL Plasma + 40 μ L 5 μ g/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 μ L dichloromethane, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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:** 17**Internal standard:** dexamethasone (23.5)**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** cortisone, hydrocortisone, prednisolone, prednisolone acetate

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: 1 mL Plasma + 150 ng dexamethasone + 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES**Guard column:** μ Bondapak/Corasil (Waters)**Column:** 300 \times 3.9 10 μ m μ Porasil (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:** 4**Internal standard:** dexamethasone (5)

OTHER SUBSTANCES**Extracted:** hydrocortisone, prednisolone

KEY WORDS

plasma; normal phase

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 + 5 mL water + 1 mL 2 μ g/mL equilenin in MeOH + 50 μ L 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.

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: 4

Internal standard: equilenin (7.5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: betamethasone, deoxycortisol, dexamethasone, hydrocortisone, prednisolone, triamcinolone

KEY WORDS

Anal.Abs. 1982, 43, 4D182; 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 + 4 mL 59.5 ng/mL triamcinolone acetonide in dichloromethane, shake at high speed for 15 min, centrifuge at 2000 rpm for 10 min, remove aqueous layer, add 5 mL saturated sodium bicarbonate solution to the organic layer, shake at high speed for 5 min, centrifuge at 2000 rpm for 10 min, remove aqueous layer. Place organic layer in a pointed tube and evaporate to dryness at 45° under a stream of nitrogen. Reconstitute with 50 µL mobile phase, inject 20 µL aliquot.

HPLC VARIABLES

Column: 10 µm Porasil

Mobile phase: Hexane:dichloromethane:ethanol:acetic acid 68.8:25:6:0.2

Flow rate: 2.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4.5

Internal standard: triamcinolone acetonide (3)

OTHER SUBSTANCES

Extracted: hydrocortisone, prednisolone

KEY WORDS

plasma; normal phase

REFERENCE

Agabeyoglu, I.T.; Wagner, J.G.; Kay, D.R. A sensitive high-pressure liquid chromatographic method for the determination of prednisone, prednisolone and hydrocortisone in plasma. *Res. Commun. Chem. Pathol. Pharmacol.*, **1980**, *28*, 163–176

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 2 μ g/mL dexamethasone in EtOH:water 10:90 + 100 μ L 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 μ L dichloromethane:EtOH:water 95:4:1, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 5 μ m Partisil silica

Mobile phase: Dichloromethane:EtOH:water 95:4:1

Flow rate: 1.5

Injection volume: 50

Detector: UV 239

CHROMATOGRAM

Retention time: 7.7

Internal standard: dexamethasone (11.5)

Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 17-hydroxyprogesterone, 6 α -methylprednisolone, prednisolone, progesterone

KEY WORDS

plasma; normal phase

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, urine

Sample preparation: Urine. Adjust pH of 5 mL urine to 7 with 500 mM pH 7 ammonium acetate buffer, extract twice with 7 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L MeOH, inject a 20 μ L aliquot. Serum. Extract 2 mL serum twice with 4 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m Lichrospher 100 RP-18

Column: 125 \times 4 5 μ m Lichrospher 100 RP-18

Mobile phase: Gradient. A was 10 mM ammonium acetate. B was MeOH. A:B from 50:50 to 0:100 over 7 min, maintain at 0:100 for 3 min.

Flow rate: 1

Injection volume: 20

Detector: MS, PE Sciex API III LC-MS-MS, heated nebulizer interface at 75 psi and 300°, auxiliary nitrogen 1.4 L/min, nitrogen curtain 1.2 L/min, negative mode ionization, dis-

charge current -3 μA , collision gas argon with 570×10^{12} molecules/sq.cm., dwell time 10 ms, step size 1 amu, m/z 327, SIM

CHROMATOGRAM

Retention time: 5.5

Internal standard: prednisone

OTHER SUBSTANCES

Extracted: triamcinolone acetonide (m/z 413)

KEY WORDS

horse; serum; prednisone is IS

REFERENCE

Koupai-Abyazani, M.R.; Yu, N.; Esaw, B.; Laviolette, B. Determination of triamcinolone acetone in equine serum and urine by liquid chromatography-atmospheric pressure ionization mass spectrometry. *J.Anal.Toxicol.*, **1995**, *19*, 182-186

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: 26

OTHER SUBSTANCES

Simultaneous: cortisone, dehydrocorticosterone, hydrocortisone, methylprednisolone, prednisolone, 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: Prepare a 25 $\mu\text{g/mL}$ 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' 0.8779

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone, cortisone, estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone, hydrocortisone, hydroxyprogesterone, lynestrenol (UV 210), medroxyprogesterone acetate, medroxyprogesterone, methandienone, methylandrostenediol (UV 210), methylprednisolone acetate, methylprednisolone, methyltestosterone, nandrolone, norethisterone, prednisolone acetate, prednisolone, pregnenolone (UV 210), progesterone, testosterone

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

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: 8.8 (prednisone acetate)

OTHER SUBSTANCES

Simultaneous: androstenedione, corticosterone, cortisol, cortisone, 11-deoxycorticosterone, 11-deoxycortisol, dexamethasone acetate, estradiol, estriol, estrone, 17α-hydroxyprogesterone, progesterone, testosterone

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. *Bio-med.Chromatogr.*, **1990**, *4*, 34–38

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 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 240

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: hydrocortisone acetate, methyltestosterone, norethindrone, prednisolone, prednisolone succinate, progesterone

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 RP-18 C18 (Brownlee)

Mobile phase: MeCN:water 40:60

Injection volume: 20

Detector: UV 254

REFERENCE

Kane, M.P.; Tsuji, K. Radiolytic degradation scheme for ⁶⁰Co-irradiated corticosteroids. *J.Pharm.Sci.*, **1983**, *72*, 30-35

SAMPLE

Matrix: solutions, tissue

Sample preparation: Buffer. Condition a 3 mL Baker C18 SPE cartridge with 400 μL MeOH. Add 100-500 μL 0.1 M pH 4.5 acetate buffer containing steroids to SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of air, reconstitute in 40-200 μL dichloromethane:MeOH 98:2, inject a 40 μL aliquot. Skin tissue. Crush tissue with 3 mL MeOH:100 mM pH 4.5 acetate buffer 20:80 using a Polytron tissue homogenizer, wash homogenizer twice with the same solution, combine all solutions, add 10 mL dichloromethane, vortex for 1 min, centrifuge at 3000 rpm for 5 min. Filter the organic phase through a column of Celite 545, evaporate to dryness under a stream of air, reconstitute in 40-200 μL dichloromethane:MeOH 98:2, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb Si-60

Mobile phase: n-Hexane:dichloromethane:MeOH:water 63.9:30:6:0.1

Flow rate: 1.2

Injection volume: 40-200

Detector: UV 240

CHROMATOGRAM

Retention time: 5.3

Internal standard: prednisone

OTHER SUBSTANCES

Extracted: betamethasone

KEY WORDS

buffer; SPE; prednisone is IS; normal phase

REFERENCE

Kubota, K.; Maibach, H.I. In vitro percutaneous permeation of betamethasone and betamethasone 17-valerate. *J.Pharm.Sci.*, **1993**, *82*, 1039-1045

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na₂HPO₄, 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: 6.3
Internal standard: methylprednisolone (9)

OTHER SUBSTANCES

Simultaneous: betamethasone, corticosterone, cortisone, dexamethasone, fluorocortisone acetate, hydroxyprogesterone, prednisolone, triamcinolone, triamcinolone acetonide
Interfering: hydrocortisone, fluorocortisone

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: Dilute, if necessary, 100 µL-1 mL urine to 1 mL with water, add 500 ng betamethasone, add to a Chem Elut high-surface-area diatomaceous earth extraction column, after 5 min elute with two 6 mL portions of ethyl acetate, combine the eluates and wash them twice with 1 mL 200 mM NaOH. Dry the organic layer over 1 g anhydrous sodium sulfate, evaporate to dryness at 30° under a stream of nitrogen, reconstitute the residue in 250 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 70 × 6 37-53 µm HC-Pellocil
Column: 250 × 4.6 5-6 µm Zorbax SIL
Mobile phase: Dichloromethane:glacial acetic acid:MeOH 91.3:7.5:1.2
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7
Internal standard: betamethasone (8.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, hydrocortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone, 20α-hydroxyprednisolone, 20β-hydroxyprednisolone, 20β-hydroxyprednisone, prednisolone

KEY WORDS

SPE

REFERENCE

Garg, V.; Jusko, W.J. Simultaneous analysis of prednisone, prednisolone and their major hydroxylated metabolites in urine by high-performance liquid chromatography. *J.Chromatogr.*, **1991**, *567*, 39–47

SAMPLE**Matrix:** urine

Sample preparation: 3 mL Urine + 1.5 µg betamethasone + 100 mg K₂HPO₄ + 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:** 60 × 4.6 3 µm Hypersil ODS

Mobile phase: Gradient. MeOH:150 mM ammonium acetate from 40:60 to 50:50 over 6 min, maintain at 50:50 for 1 min, to 60:40 over 3 min, maintain at 60:40 for 5 min.

Flow rate: 0.8**Injection volume:** 15

Detector: MS, Hewlett-Packard HP 5988A, vaporizer probe 92° decreased to 89° over 6 min, decreased to 86° over 3 min, maintain at 86° for 5 min, ion source 276°, emission current 150 µA, electron energy 955 eV, positive ion mode, filament on

CHROMATOGRAM**Retention time:** 4.2**Internal standard:** betamethasone (7)**Limit of detection:** 1-5 ng (SIM)

OTHER SUBSTANCES

Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 11α-hydroxyprogesterone, prednisolone, 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 + 1.5 µg betamethasone + 100 mg K₂HPO₄ + 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. MeOH: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:** 3.5

Internal standard: betamethasone

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 11 α -hydroxyprogesterone, prednisolone, 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: Dilute 0.1-1 mL urine to 1 mL with water if necessary, add 50 μ L 10 μ g/mL dexamethasone in MeOH, vortex, add to a Chem-Elut cartridge (cat. no. 1003), allow to stand for 5 min, elute twice with 5 mL portions of ethyl acetate (which are first used to rinse the sample tube) at 5 min intervals, wash eluate twice with 1 mL 200 mM NaOH, add 1 g anhydrous sodium sulfate, let stand for 30 min. Evaporate the organic phase at 30 $^{\circ}$ under a stream of nitrogen. Reconstitute the residue in 250 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Beckman Si column

Mobile phase: Dichloromethane:MeOH:THF:glacial acetic acid 96.9:2:1:10.1

Flow rate: 1.3

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 6.4

Internal standard: dexamethasone (8.0)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: 6 β -hydroxyprednisolone, prednisolone

KEY WORDS

normal phase;SPE

REFERENCE

Teng, R.-L.; Benet, L.Z. Simultaneous measurement of prednisone, prednisolone and 6 β -hydroxyprednisolone in urine by high-performance liquid chromatography using dexamethasone as the internal standard. *J.Chromatogr.*, **1989**, *493*, 421–423

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Valvo, L.; Paris, A.; Savella, A.L.; Gallinella, B.; Ciranni Signoretti, E. General high-performance liquid chromatographic procedures for the rapid screening of natural and synthetic corticosteroids. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 805–810 [gradient; reverse phase; normal phase; also beclomethasone, beclomethasone 17,21-dipropionate, betamethasone, betamethasone 21-acetate, betamethasone 17,21-dipropionate, betamethasone 21-disodium phosphate, betamethasone 17-valerate, cortisone, cortisone 21-acetate, 11-deoxycorticosterone 21-acetate, dexamethasone, dexamethasone 21-acetate, dexamethasone 21-disodium phosphate, flucinolone, flucinolone acetonide, 9 α -fluorohydrocortisone 21-acetate, 9 α -fluorohydrocortisone, 9 α -fluoroprednisolone, 9 α -fluoroprednisolone 21-acetate, hydrocortisone, hydrocortisone 21-acetate, hydrocortisone 21-hemisuccinate, 6 α -methylprednisolone, 6 α -methylprednisolone 21-acetate, 6 α -methylprednisolone 21-sodium succinate, prednisolone,

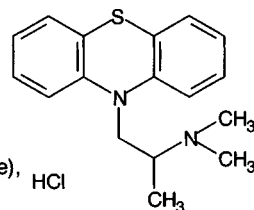
- prednisolone 21-acetate, prednisolone 21-disodium phosphate, prednisolone 21-pivalate, prednisolone 21-sodium succinate, triamcinolone, triamcinolone acetonide]
- Santos-Montes, A.; Gasco-Lopez, A.I.; Izquierdo-Hornillos, R. Optimization of the high-performance liquid chromatographic separation of a mixture of natural and synthetic corticosteroids. *J.Chromatogr.*, **1993**, *620*, 15–23 [urine; column temp 30; extracted betamethasone, corticosterone, cortisol, deoxycorticosterone, dexamethasone, fluorocortisone, hydrocortisone, hydroxyprogesterone, methylprednisolone, prednisolone, triamcinolone]
- Ulrich, B.; Frey, F.J.; Speck, R.F.; Frey, B.M. Pharmacokinetics/pharmacodynamics of ketoconazole-prednisolone interaction. *J.Pharmacol.Exp.Ther.*, **1992**, *260*, 487–490 [mouse; plasma; dexamethasone (IS); LOD 10 ng/mL; extracted prednisolone]
- Cannell, G.R.; Mortimer, R.H.; Maguire, D.J.; Addison, R.S. Liquid chromatographic analysis of prednisolone, prednisone and their 20-reduced metabolites in perfusion media. *J.Chromatogr.*, **1991**, *563*, 341–347
- McBride, J.H.; Rodgerson, D.O.; Park, S.S.; Reyes, A.F. Rapid liquid-chromatographic method for simultaneous determination of plasma prednisone, prednisolone, and cortisol in pediatric renal-transplant recipients. *Clin.Chem.*, **1991**, *37*, 643–646
- Alvinerie, M.; Sutra, J.F.; Galtier, P.; Houin, G.; Toutain, P.L. Simultaneous measurement of prednisone, prednisolone and hydrocortisone in plasma by high performance liquid chromatography. *Ann.Biol.Clin.(Paris)*, **1990**, *48*, 87–90
- Huber, F.; Wiedemann, M.; Heinrich, G.; Salama, Z.; Jaeger, H. Development of a high performance liquid chromatography method for the simultaneous measurement of prednisone and prednisolone. *Arzneimittelforschung*, **1990**, *40*, 926–931
- Lasic, S.; Bobarevic, N.; Nikolin, B. Simultaneous determination of prednisone, prednisolone, cortisol and dexamethasone in plasma by high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1989**, *7*, 777–782
- Frey, B.M.; Frey, F.J. Simultaneous measurement of prednisone, prednisolone and 6 β -hydroxyprednisolone in urine by high-performance liquid chromatography coupled with a radioactivity detector. *J.Chromatogr.*, **1982**, *229*, 283–292
- Ui, T.; Mitsunaga, M.; Tanaka, T.; Horiguchi, M. Determination of prednisone and prednisolone in human serum by high-performance liquid chromatography—especially on impaired conversion of corticosteroids in patients with chronic liver disease. *J.Chromatogr.*, **1982**, *239*, 711–716
- Rocci, M.L., Jr.; Jusko, W.J. Analysis of prednisone, prednisolone and their 20 β -hydroxylated metabolites by high-performance liquid chromatography. *J.Chromatogr.*, **1981**, *224*, 221–227 [perfusate; urine; 6 β -hydroxycortisol (IS); triamcinolone (IS); LOD 10 ng/mL; extracted 20 β -hydroxyprednisolone, 20 β -hydroxyprednisone, prednisolone]
- Rose, J.Q.; Jusko, W.J. Corticosteroid analysis in biological fluids by high-performance liquid chromatography. *J.Chromatogr.*, **1979**, *162*, 273–280 [plasma; urine; saliva; extracted hydrocortisone, prednisolone; dexamethasone (IS); LOD 5 ng/mL; pharmacokinetics; simultaneous beclomethasone, methylprednisolone, triamcinolone acetonide]

Promethazine

Molecular formula: C₁₇H₂₀N₂S

Molecular weight: 284.4

CAS Registry No.: 60-87-7 (promethazine), 58-33-3 (promethazine hydrochloride), 17693-51-5 (promethazine teoclate)



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)) KH₂PO₄ 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: 7.46

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrridine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephesisin, mephentermine, mepivacaine, metapramine, metformin, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, ox-

azepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, propranolol, protriptyline, pyrimethamine, quinidine, quinine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: amoxapine, betaxolol, cyproheptadine, dextropropoxyphene, fenopropfen, loxapine, methadone, opipramol, promethazine, propafenone, quinupramine, thioproperazine

KEY WORDS

whole blood; plasma

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 + 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 μ L MeCN, inject a 30 μ L aliquot (store at 5°).

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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 +0.25 V, sample electrode +0.5 V

CHROMATOGRAM

Retention time: 9

Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: chlorprothixene

Interfering: methotrimeprazine (levomepromazine)

KEY WORDS

serum; work under yellow light; 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: 1 mL Serum + 100 μ L 100 ng/mL chlorpromazine, vortex at low speed for 10 s, add 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 μ L 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 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m 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: 7.7

Internal standard: chlorpromazine (10.2)

Limit of quantitation: 0.2 ng/mL

KEY WORDS

serum

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: 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 10 μ m 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: 5.7

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, butaperazine, carphenazine, chlorpromazine, promazine, thioridazine, trifluoperazine, trimeprazine

Simultaneous: acetophenazine, benztropine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, thiothixene, triflupromazine, trihexyphenidyl

KEY WORDS

plasma; whole blood

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

Sample preparation: Plasma. 1-5 mL Plasma + 1 mL 1 M NaOH, extract with mixed hexanes for 30 min, centrifuge. Remove a 9 mL aliquot of the hexane layer and evaporate it to dryness under a stream of nitrogen at 30°, dissolve residue in 100 µL mobile phase, inject a 50 µL aliquot. Whole blood. 10 mL Whole blood + 1 mL 1 M NaOH, extract with 15 mL mixed hexanes for 1 h. Remove an aliquot of the hexane layer and evaporate it to dryness, reconstitute the residue in 1 mL 100 mM HCl, extract with 5 mL chloroform by vortexing for 1 min, centrifuge. Remove a 4.5 mL aliquot of the chloroform layer, evaporate to dryness, dissolve in 10 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 10 µm Micropak CN

Mobile phase: MeCN:5 mM ammonium acetate 90:10 (vary ammonium acetate concentration to achieve best separation)

Flow rate: 2.5

Injection volume: 10-50

Detector: UV 254; E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 13.4

Limit of detection: 10 ng/mL (UV); 0.1 ng/mL (E)

OTHER SUBSTANCES

Extracted: acetophenazine, amitriptyline, benztrapine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, piperacetazine, orphenadrine, promazine, 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 or serum + 100 mg sodium carbonate, vortex for 3 s, add 10 mL 3 ng/mL triflupromazine in hexane:MeOH 99.7:0.3, shake for 15 min, centrifuge at 2000 rpm for 5 min. Remove 9.3 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 50 µL ethyl acetate, add 25 µL trichloroethyl chloroformate, vortex, heat at 120° for 20 min, cool. Evaporate to dryness under a stream of air at 40°, reconstitute the residue in 100 µL MeOH, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 300 × 4 MCH-10 reversed-phase (Varian)

Mobile phase: MeOH:water 84:16

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 4.2

Internal standard: triflupromazine (6)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: chlorpromazine, chlorprothixene, phenothiazine-5-oxide, promazine, trimepazine

Interfering: phenothiazine

KEY WORDS

plasma; serum; derivatization

REFERENCE

Wallace, J.E.; Shimek, E.L., Jr.; Harris, S.C.; Stavchansky, S. Determination of promethazine in serum by liquid chromatography. *Clin.Chem.*, **1981**, *27*, 253-255

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Condition a 3 mL Varian Certify SPE cartridge with two cartridge volumes of MeOH and two cartridge volumes of 100 mM pH 6.0 KH_2PO_4 . 5 mL Urine + 50 μL 1 mg/mL chlorpromazine in water + 5 mL water + 1 mL phosphate buffer, vortex for 1 min, add to the SPE cartridge, rinse the tube with 1 mL phosphate buffer, add the rinse to the SPE cartridge, dry under vacuum for 2-5 min, wash with three 500 μL portions of MeOH:concentrated HCl 99:1 and two 500 μL portions of 1 M acetic acid (allow to dry after each wash), elute with six 1 mL portions of 2% ammonium hydroxide in EtOH. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 1 mL mobile phase, vortex for 3 min, inject an aliquot. Serum. Condition a 3 mL Varian Certify SPE cartridge with two cartridge volumes of MeOH and two cartridge volumes of 100 mM pH 6.0 KH_2PO_4 . 3 mL Serum + 50 μL 1 mg/mL chlorpromazine in water + 5 mL water + 1 mL phosphate buffer, vortex for 1 min, add to the SPE cartridge, rinse the tube with 1 mL phosphate buffer, add the rinse to the SPE cartridge, dry under vacuum for 2-5 min, wash with three 500 μL portions of MeOH:concentrated HCl 99:1 and two 500 μL portions of 1 M acetic acid (allow to dry after each wash), elute with six 1 mL portions of 2% ammonium hydroxide in EtOH. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, vortex for 3 min, inject an aliquot. (To determine low levels of promethazine enantiomers the residue was reconstituted with 300 μL mobile phase containing 5 mg/mL enantiomer. The amount of promethazine in the serum was calculated by the increase in peak area over the control value.)

HPLC VARIABLES

Column: 100 \times 4.6 5 μm KK-CARNU (α -R-naphthyl)urea (YMC)

Mobile phase: Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 40:15:10:0.1

Flow rate: 1

Injection volume: 20

Detector: F ex 254 em 280 (filter)

CHROMATOGRAM

Retention time: 12.40 (R-+), 13.28 (S-(-))

Internal standard: chlorpromazine (19.13)

Limit of detection: 400 ng/mL

KEY WORDS

chiral; serum; SPE

REFERENCE

Ponder, G.W.; Stewart, J.T. A liquid chromatographic method for the determination of promethazine enantiomers in human urine and serum using solid-phase extraction and fluorescence detection. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1161–1166

SAMPLE**Matrix:** bulk**Sample preparation:** Prepare a solution in the mobile phase.

HPLC VARIABLES**Column:** 250 × 4.6 Cyclobond I (Advanced Separation Technologies)**Mobile phase:** MeOH:buffer 17:83 (Buffer was 2.5% triethylamine acetate, pH 5.50.)**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 10.2

OTHER SUBSTANCES**Simultaneous:** isopromethazine

REFERENCE

Piperaki, S.; Perakis, A.; Parissi-Poulou, M. Liquid chromatographic retention behaviour and separation of promethazine and isopromethazine on β -cyclodextrin bonded-phase column. *J.Chromatogr.A*, **1994**, *660*, 339–350

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute with saline, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 μ Bondapak C18**Mobile phase:** MeCN:100 mM K₂HPO₄ 33:67 adjusted to a final pH of 4.4 with phosphoric acid**Flow rate:** 1.7**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5.5

OTHER SUBSTANCES**Interfering:** midazolam

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: formulations

Sample preparation: Sonicate in mobile phase, dilute the clear supernatant to a concentration of 3-10 $\mu\text{g/mL}$, filter (0.5 μm), inject an aliquot.

HPLC VARIABLES

Column: 250 \times 2.6 10 μm Porasil silica gel (Perkin-Elmer)

Mobile phase: MeOH containing 0.02% NH_4OH : dichloromethane 20:80

Flow rate: 1

Injection volume: 20

Detector: UV 310

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: aminophylline

KEY WORDS

suppositories; normal phase

REFERENCE

Kountourellis, J.E.; Raptouli, A.; Georgarakis, M. Simultaneous determination of aminophylline and promethazine in suppositories by high-performance liquid chromatography. *Pharmazie*, **1986**, *41*, 600-601

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, injections. 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 \times 3.9 10 μm $\mu\text{Bondapak CN}$

Mobile phase: MeOH:3 mM ammonium acetate 90:10

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 3.3

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, propriptyline, pyrilamine, pyrimethamine, tripeleminamine

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 × 4.6 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 254

CHROMATOGRAM

Retention time: 6.4, 6.8 (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

HPLC VARIABLES

Column: 100 × 4.5 μm CHIRAL-AGP (ChromTech)

Mobile phase: MeCN:59 mM pH 4.0 acetate buffer 1:99

Flow rate: 0.9

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: k' 7.68, 10.1 (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 α₁-acid glycoprotein column. *J.Chromatogr.A*, **1995**, *694*, 57–69

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES

Column: 100 × 4.7 μm Hypercarb (Shandon)

Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH

Column temperature: 17

Injection volume: 20

Detector: UV 270

CHROMATOGRAM**Retention time:** k' 58 (first enantiomer)

KEY WORDSchiral; $\alpha = 1.23$

REFERENCEHuynh, N.-H.; Karlsson, A.; Pettersson, C. Enantiomeric separation of basic drugs using N-benzyloxy-carbonylglycyl-L-proline as counter ion in methanol. *J.Chromatogr.A*, **1995**, *705*, 275-287

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 \times 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' 32.21

OTHER SUBSTANCES**Also analyzed:** acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimeti-dine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxa-zosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pin-dolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrillamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelen-namine, triprolidine, tymazoline, UK-14,304

REFERENCEKaliszan, 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 \times 4.6 5 μm Supelcosil LC-DP (A) or 250 \times 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:** 13.20 (A), 6.40 (B)

OTHER SUBSTANCES**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atro-

pine, 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, fenopropfen, 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, methyl dopa, 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, pimizole, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocanide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also some 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: Make up a 500 ng/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Chiralcel-OJ

Mobile phase: Hexane:EtOH 50:50

Flow rate: 0.8

Injection volume: 100

Detector: F ex 254 em 280 (filter)

CHROMATOGRAM

Retention time: 8.30, 9.93 (enantiomers)

KEY WORDS

chiral

REFERENCE

Ponder, G.W.; Butram, S.L.; Adams, A.G.; Ramanathan, C.S.; Stewart, J.T. Resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on selected chiral stationary phases using high-performance liquid chromatography. *J.Chromatogr.A*, **1995**, *692*, 173–182

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

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m 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:** 2.49

OTHER SUBSTANCES**Simultaneous:** acetophenazine, carphenazine, deserpidine, ethopropazine, methotrimeprazine, methotrimeprazine, perphenazine, promazine, reserpine, thiothixene, triflupromazine**Interfering:** chlorprothixene, propiomazine

KEY WORDS

SFC; pressure 200 bar

REFERENCEBerger, 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 \times 4.6 amylose tris(3,5-dichlorophenylcarbamate)**Mobile phase:** Hexane:isopropanol 98:2**Flow rate:** 0.5**Detector:** UV

CHROMATOGRAM**Retention time:** k' 1.84 (of first (+) enantiomer)

KEY WORDSchiral; α = 1.24

REFERENCEOkamoto, 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:** Dissolve in MeOH:water 1:1 at a concentration of 50 μ g/mL, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μ m μ Bondapak 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: 11

OTHER SUBSTANCES

Simultaneous: 8-chlorotheophylline, diphenhydramine, diphenylpyraline

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 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: 5.5

OTHER SUBSTANCES

Simultaneous: acetophenazine, butaperazine, chlorpromazine, mesoridazine, prochlorperazine, thiethylperazine, thioridazine

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 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: 5.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotanine, 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, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phen butrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phen tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pin dolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piri tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin tane, promazine, pronethalol, propoperidine, propiomazine, propranolol, prothipendyl, pro triptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, raniti dine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, tri fluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimetho prim, trimipramine, tripeleppamine, 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

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Koytchev, R.; Alken, R.-G.; Kirkov, V.; Neshev, G.; Vagaday, M.; Kunter, U. Absolute Bioverfügbarkeit von Chlorpromazin, Promazin und Promethazin [Absolute bioavailability of chlorpromazine, promazine and promethazine]. *Arzneimittelforschung*, **1994**, *44*, 121–125 [column temp 40; LOD 1-2 ng/mL; serum; pharmacokinetics]

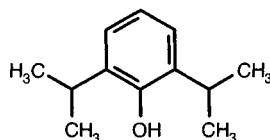
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- Schill, G.; Wainer, I.W.; Barkan, S.A. Chiral separation of cationic drugs on an α 1-acid glycoprotein bonded stationary phase. *J.Liq.Chromatogr.*, **1986**, *9*, 641–666 [also atropine, bromdiphenhydramine, brompheniramine, bupivacaine, butorphanol, carbinoxamine, chlorpheniramine, elidinium, cocaine, cyclopentolate, dimethindene, diperidone, disopyramide, doxylamine, ephedrine, homatropine, labetalol, labetalol A, labetalol B, mepensolate, mepivacaine, methadone, methorphan, methylatropine, methylhomatropine, methylphenidate, metoprolol, nadolol, nadolol A, nadolol B, oxprenolol, oxyphencyclimine, phenmetrazine, phenoxybenzamine, pronethalol, propoxyphene, propranolol, pseudoephedrine, terbutaline, tocinide, tridihexethyl]
- Leelavathi, D.E.; Dressler, D.E.; Soffer, E.F.; Yachetti, S.D.; Knowles, J.A. Determination of promethazine in human plasma by automated high-performance liquid chromatography with electrochemical detection and by gas chromatography-mass spectrometry. *J.Chromatogr.*, **1985**, *339*, 105–115 [LOD 0.1 ng/mL; column-switching; thioridazine (IS)]
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- Stavchansky, S.; Wallace, J.; Chue, M.; Newburger, J. High pressure liquid chromatographic determination of promethazine hydrochloride in the presence of its thermal and photolytic degradation products: A stability indicating assay. *J.Liq.Chromatogr.*, **1983**, *6*, 1333–1344 [promazine (IS); column temp 45]
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- 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 [post-column reaction; fluorescence detection; also demoxepam, chlorpromazine, methotrimeprazine, nedaltran]
- Wallace, J.E.; Shimek, E.L.J.; Stavchansky, S.; Harris, S.C. Determination of promethazine and other phenothiazine compounds by liquid chromatography with electrochemical detection. *Anal.Chem.*, **1981**, *53*, 960–962
- DiGregorio, G.J.; Ruch, E. Human and whole blood and parotid saliva concentrations of oral and intramuscular promethazine. *J.Pharm.Sci.*, **1980**, *69*, 1457–1461 [promazine (IS); LOQ 2.5 ng/mL (whole blood); LOQ 0.5 ng/mL (saliva)]

Propofol

Molecular formula: C₁₂H₁₈O

Molecular weight: 178.3

CAS Registry No.: 2078-54-8



SAMPLE

Matrix: blood

Sample preparation: 1 ml Whole blood + 20 μ L thymol in MeOH + 1 mL 100 mM NaH₂PO₄ + 5 mL cyclohexane, shake at 200 rpm for 15 min, centrifuge at 1200 g for 5 min. Remove 4.5 mL of the organic layer and add it to 50 μ L 1.875% tetraethylammonium hydroxide in EtOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4 C18

Mobile phase: MeCN:buffer 67:33 (Buffer was water adjusted to pH 4.0 with acetic acid.)

Detector: UV

CHROMATOGRAM

Retention time: 13

Internal standard: thymol (5-methyl-2-isopropylphenol) (8)

Limit of quantitation: 500 ng/mL

KEY WORDS

whole blood; pharmacokinetics

REFERENCE

Dawidowicz, A.L.; Fijalkowska, A. Determination of propofol in blood by HPLC. Comparison of the extraction and precipitation methods. *J.Chromatogr.Sci.*, **1995**, *33*, 377-382

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Supelclean LC-18 SPE cartridge (Supelco) with two 1 mL portions of MeCN and two 1 mL portions of water. 50 μ L Whole blood + 25 ng thymol + 200 μ L 100 mM KH₂PO₄, mix, add to the SPE cartridge, wash with two 1 mL portions of water, elute with MeCN. Discard the first 50 μ L eluate and collect the next 600 μ L, evaporate to about 200 μ L under a stream of nitrogen, inject a 30 μ L aliquot.

HPLC VARIABLES

Guard column: Chrompack RP guard column

Column: 100 \times 4.6 5 μ m Spherisorb S5ODS2

Mobile phase: MeCN:water:85% orthophosphoric acid 46:54:0.1

Flow rate: 1

Injection volume: 30

Detector: F ex 276 em 310

CHROMATOGRAM

Internal standard: thymol

Limit of quantitation: 50 ng/mL

KEY WORDS

whole blood; SPE; pharmacokinetics

REFERENCE

Lee, H.-S.; Khoo, Y.-M.; Chua, B.-C.; Ng, A.S.-B.; Tan, S.S.-W.; Chew, S.-L. Pharmacokinetics of propofol infusion in Asian patients undergoing coronary artery bypass grafting. *Ther.Drug Monit.*, **1995**, *17*, 336-341

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 500 μ L 20 μ g/mL dibutyl phthalate in MeCN:65% perchloric acid 67:33, vortex for 1 min, centrifuge at 1150 g for 5 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Spherisorb C18

Mobile phase: MeCN:water:acetic acid 67:33:0.04, pH 4.0

Flow rate: 1.5

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Internal standard: dibutyl phthalate

Limit of detection: 100 ng/mL

KEY WORDS

serum

REFERENCE

Pavan, I.; Buglione, E.; Massiccio, M.; Gregoretti, C.; Burbi, L.; Berardino, M. Monitoring propofol serum levels by rapid and sensitive reversed-phase high-performance liquid chromatography during prolonged sedation in ICU patients. *J.Chromatogr.Sci.*, **1992**, *30*, 164-166

SAMPLE

Matrix: blood, urine

Sample preparation: Inject a 10-400 μ L aliquot of serum or urine onto column A with mobile phase A and elute to waste, 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 20 \times 4 37-50 μ m Bondapak C18/Corasil; B 125 \times 4 5 μ m LiChrosorb RP-18

Mobile phase: A 10 mM pH 7.88 KH_2PO_4 ; B MeCN:10 mM pH 7.88 KH_2PO_4 , 70:30

Flow rate: A 1.5; B 1.3

Injection volume: 10-400

Detector: F ex 276 em 310

CHROMATOGRAM

Retention time: 5

Limit of quantitation: 5 ng/mL

KEY WORDS

serum; column-switching

REFERENCE

Altmayer, P.; Büch, U.; Büch, H.P.; Larsen, R. Rapid and sensitive pre-column extraction high-performance liquid chromatographic assay for propofol in biological fluids. *J.Chromatogr.*, **1993**, *612*, 326-330

SAMPLE

Matrix: formulations

Sample preparation: Filter (0.45 μm). Add an aliquot to an aliquot of 1 mg/mL thymol, make up to 3 mL with MeOH, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Hypersil ODS C18

Mobile phase: MeCN:MeOH:water 55:10:35

Flow rate: 2

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 7.5

Internal standard: thymol (4.0)

KEY WORDS

stability-indicating; parenteral nutrient solutions

REFERENCE

Bhatt-Mehta, V.; Paglia, R.E.; Rosen, D.A. Stability of propofol with parenteral nutrient solutions during simulated Y-site injection. *Am.J.Health-Syst.Pharm.*, **1995**, 52, 192–196

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Supelcosil LC-DP (A) or 250 \times 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: 10.07 (A), 15.24 (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, fenopropfen, 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, methyl dopa, 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, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazone, 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

some 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

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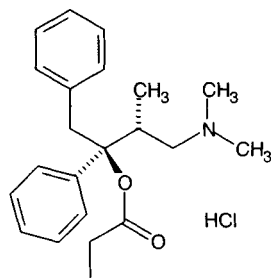
- Fan, S.Z.; Yu, H.Y.; Chen, Y.L.; Liu, C.C. Propofol concentration monitoring in plasma or whole blood by gas chromatography and high-performance liquid chromatography. *Anesth.Analg.*, **1995**, 81, 175–178
- Bailey, L.C.; Tang, K.T.; Rogozinski, B.A. The determination of 2,6-diisopropylphenol (propofol) in an oil in water emulsion dosage form by high-performance liquid chromatography and by second derivative UV spectroscopy. *J.Pharm.Biomed.Anal.*, **1991**, 9, 501–506
- Chan, K.; So, A.P. The measurement of propofol in human blood samples by liquid chromatography. *Methods Find.Exp.Clin.Pharmacol.*, **1990**, 12, 135–139
- Mazzi, G.; Schinella, M. Simple and practical high-performance liquid chromatographic assay of propofol in human blood by phenyl column chromatography with electrochemical detection. *J.Chromatogr.*, **1990**, 528, 537–541

Propoxyphene

Molecular formula: C₂₂H₂₉NO₂

Molecular weight: 339.5

CAS Registry No.: 469-62-5 (propoxyphene), 1639-60-7 (propoxyphene hydrochloride), 26570-10-5 (propoxyphene napsylate monohydrate), 55557-30-7 (l-form propoxyphene napsylate monohydrate), 2338-37-6 (l form propoxyphene), 17140-78-2 (l-form propoxyphene napsylate anhydrous)



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)) KH₂PO₄ 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: 7.24

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carboxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlorfenezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrridine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, mida-

zolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thio-pental, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: betaxolol, dextromoramide, dextropropoxyphene, dosulepine, fenoprofen, loxapine, methadone, promethazine, propafenone, thioproperazine

KEY WORDS

whole blood; plasma

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 μ L MeOH, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m 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 340-266, collision offset -6 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 μ A, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM

Retention time: 7.10 (dextropropoxyphene)

Limit of detection: 50 μ g

OTHER SUBSTANCES

Extracted: benperidol, dextromoramide, droperidol, haloperidol, methadone, penfluridol, pimozide, pipamperidone

KEY WORDS

SPE; LC/MS

REFERENCE

Verweij, A.M.A.; Hordijk, M.L.; Lipman, P.J.L. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrphenone, or diphenylbutylpiperidine groups in whole blood. *J.Anal.Toxicol.*, **1995**, *19*, 65-68

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 the SPE cartridge, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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: 5.2

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propranolol, protriptyline, quinidine, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylcegonine, 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, methylclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocanide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: maprotiline, methadone, nordiazepam, norfluoxetine, temazepam

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 + 20 μ L 100 μ g/mL methadone in MeOH + 1 mL pH 11 Normex buffer (Carlo Erba), vortex for 20 s, add to a 3 mL Extrelut cartridge, wait for 10 min, elute with diethyl ether:dichloromethane 70:30. Add the eluate to 100 μ L 10 mM HCl and evaporate it at 40° under a stream of nitrogen. Wash the residual acid solution with 3 mL diethyl ether, inject a 40 μ L aliquot of the aqueous phase.

HPLC VARIABLES**Guard column:** 5 \times 6 μ Bondapak C18 Guard-Pak**Column:** 300 \times 3.9 10 μ m μ Bondapak C18**Mobile phase:** MeCN:buffer 75:25 (Buffer was 0.1% acetic acid in 10 mM ammonium acetate.)**Flow rate:** 1.5**Injection volume:** 40**Detector:** UV 215

CHROMATOGRAM**Retention time:** 13.42**Internal standard:** methadone (16.40)**Limit of detection:** 40 ng/mL

OTHER SUBSTANCES**Extracted:** dextromoramide, norpropoxyphene**Simultaneous:** alimemazine, amitriptyline, amphetamine, bupivacaine, buprenorphine, clomipramine, cocaine, codeine, ethylmorphine, glafenine, meperidine, methamphetamine, methotrimeprazine, morphine, norcodeine, nortriptyline, pentazocine**Noninterfering:** acetaminophen, aspirin, barbiturates, benzoylecgonine, caffeine, diazepam, flunitrazepam, lidocaine, lorazepam, nalorphine, naloxone, nitrazepam, nordiazepam, norflunitrazepam, normorphine, oxazepam, prazepam, triazolam, zolpidem**Interfering:** cyamemazine, desipramine, imipramine

KEY WORDS

whole blood

REFERENCERop, P.P.; Grimaldi, F.; Bresson, M.; Fornaris, M.; Viala, A. Simultaneous determination of dextromoramide, propoxyphene and norpropoxyphene in necropsic whole blood by liquid chromatography. *J.Chromatogr.*, **1993**, *615*, 357–364

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Plasma + 20 μ L 20 μ M IS in water + 300 μ L 100 mM pH 10.0 sodium carbonate buffer + 4 mL n-hexane:diethyl ether:n-butanol 70:25:5, rotate gently for 10 min, centrifuge at 3200 g at 2° for 10 min. Remove the organic layer and add it to 250 μ L pH 2.0 phosphate buffer, vortex for 15 s, centrifuge, inject 175 μ L of the aqueous layer.

HPLC VARIABLES**Column:** 100 \times 4.6 YMC S-3 120A ODS (YMC)**Mobile phase:** MeCN:buffer 39.5:60.5 + 0.2 mM N, N-dimethyloctylamine + 1 mM sodium decyl sulfate (Buffer was 50 mM NaH₂PO₄ adjusted to pH 2.0 with orthophosphoric acid.)**Flow rate:** 1.3**Injection volume:** 175**Detector:** UV 210

CHROMATOGRAM**Retention time:** 6**Internal standard:** 1-benzyl-3-methylethylamino-2-methyl-1-phenylpropyl propionate picrate (7.5)**Limit of quantitation:** 2 nM

OTHER SUBSTANCES**Extracted:** norpropoxyphene

KEY WORDSplasma

REFERENCEPetterson, K.J.; Nilsson, L.B. Determination of dextropropoxyphene and norpropoxyphene in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1992**, *581*, 161-164

SAMPLE**Matrix:** blood**Sample preparation:** 1 mL Serum + 200 ng doxepin or desipramine + 500 μ L 2% pH 9.5 sodium tetraborate + 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 μ L 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES**Column:** 300 \times 4 μ Bondapak 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:** 18.6**Internal standard:** doxepin (12.2), desipramine (14.2)**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** cocaine, dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, pentazocine**Simultaneous:** amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDSserum

REFERENCEHackett, L.P.; Duscii, 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, CSF**Sample preparation:** 200 μ L Serum, plasma, or CSF + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 HCl 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 KH₂PO₄. 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: 6.96

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

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

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, CSF, gastric fluid, 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 hydrochloride 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 KH₂PO₄. 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: 6.90

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazeopoxide, 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, 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, milk

Sample preparation: Adjust 0.2-1 mL plasma or breast milk to 1 mL with blank biological fluid if necessary, add 50 μ L 12 μ g/mL IS in EtOH, add 500 μ L 1 M pH 9.80 carbonate: bicarbonate buffer, add 10 mL n-butyl chloride, shake at high speed on a reciprocating shaker for 15 min, centrifuge at 2000 g for 5 min. Remove the organic phase and add it to 5 mL 200 mM HCl, shake at high speed on a reciprocating shaker for 15 min, centrifuge at 2000 g for 5 min. Remove the aqueous phase and wash it with 5 mL ether. Add 500 μ L 4 M NaOH to the aqueous phase, add 10 mL chloroform, shake at high speed on a reciprocating shaker for 15 min, centrifuge at 2000 g for 5 min. Remove the organic phase and evaporate it to dryness under nitrogen at 55°, dissolve the residue in 200 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:2 mM sulfuric acid 50:50

Flow rate: 1.5

Injection volume: 100

Detector: UV 205

CHROMATOGRAM

Retention time: 5.7

Internal standard: 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) (8.8)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: norpropoxyphene

KEY WORDS

plasma; breast

REFERENCE

Kunka, R.L.; Yong, C.-L.; Ladik, C.F.; Bates, T.R. Liquid chromatographic determination of propoxyphene and norpropoxyphene in plasma and breast milk. *J.Pharm.Sci.*, **1985**, *74*, 103–104

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 1 mL Whole blood or serum + 1 μ g cyanopramine + 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 μ L 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μ L aliquot of the aqueous layer. Tissue.

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 NaH₂PO₄:diethylamine 40:57.5:2.5

Flow rate: 2

Injection volume: 30

Detector: UV 220

CHROMATOGRAM

Retention time: 12.76

Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, benzotropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranlycypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphethidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: mesoridazine

KEY WORDS

whole blood; serum; 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: 2 mL Blood or 250 mg liver (homogenized with 3 parts water) or 500 mg brain (homogenized with 3 parts water) + 2 µg SKF-525-A + 1.5 mL pH 9.5 ammonium carbonate/ammonium hydroxide buffer + 10 mL hexane:isopropanol 99:1, rotate at 10 rpm for 10 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and add it to 2.5 mL 0.25 M sulfuric acid, rotate for 5 min, centrifuge at 1500 rpm for 5 min. Remove the aqueous layer and add concentrated ammonium hydroxide to make the pH 9.5, add 1.5 mL chloroform, vortex for 15 s, centrifuge at 1500 rpm for 5 min. Remove the organic layer and add 1 drop of 1% HCl in MeOH, evaporate to dryness at 50° under vacuum, reconstitute with 200 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 30 × 2.1 Whatman C18 pellicular

Column: 250 × 4.6 Spherisorb S-5-ODS

Mobile phase: MeCN:MeOH:buffer 48:4:48 (Buffer was 1980 mL water + 20 mL 85% phosphoric acid + 3.7 mL methanesulfonic acid adjusted to pH 3.0 with 5 M NaOH.)

Column temperature: 60

Flow rate: 2

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 6.0

Internal standard: SKF-525-A (11.2)

Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: N-desmethyldiazepam, diazepam, methadone, norpropoxyphene

KEY WORDS

liver; brain

REFERENCE

Rio, J.; Hodnett, N.; Bidanset, J.H. The determination of propoxyphene, norpropoxyphene, and methadone in postmortem blood and tissues by high-performance liquid chromatography. *J.Anal.Toxicol.*, 1987, 11, 222-224

SAMPLE

Matrix: formulations

Sample preparation: Add one tablet to 10 mL MeOH and 80 mL dichloromethane, sonicate for 5 min, dilute to 100 mL with dichloromethane, dilute a 2 mL aliquot to 25 mL with mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Supelcosil

Column: 33 \times 4.6 3 μ m Supelcosil

Mobile phase: Dichloromethane:3.33% ammonium hydroxide in MeOH 98.5:1.5

Flow rate: 2

Injection volume: 10

Detector: UV 244

CHROMATOGRAM

Retention time: 2

Limit of detection: 2500 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen

Noninterfering: impurities, 4-aminophenol, p-hydroxyacetophenone, p-nitrophenol

KEY WORDS

tablets; normal phase

REFERENCE

Ascah, T.L.; Hunter, B.T. Simultaneous high-performance liquid chromatographic determination of propoxyphene and acetaminophen in pharmaceutical preparations. *J.Chromatogr.*, 1988, 455, 279-289

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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 1 L water.

thylamine 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, bromazepam, 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, 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, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidin, 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, pyrillamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleppamine, 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: 300 × 3.9 μBondapak C18

Mobile phase: MeCN: 10 mM KH₂PO₄ + 5 mM 1-decanesulfonic acid:heptylamine 50:50:0.1, pH adjusted to 7.9 with 85% phosphoric acid

Flow rate: 2

Injection volume: 10

Detector: UV 220

CHROMATOGRAM

Retention time: 13.6

Internal standard: papaverine (2.7)

Limit of detection: 50 ng/mL

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: 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, bamethane, 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, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, iso-xsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, 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, pimozone, 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, tranlycypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleannamine, 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 at a concentration of 1 mg/mL, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 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: 1.57

OTHER SUBSTANCES

Simultaneous: acetylcodeine, amphetamine, benzylmorphine, bromo-STP, chlorphentermine, codeine, codeine-N-oxide, diamorphine, dihydrocodeine, dihydromorphine, dimethylamphetamine, dipipanone, ephedrine, epinephrine, ethoheptazine, ethylmorphine, fencamfamin, fenfluramine, hydrocodone, 4-hydroxyamphetamine, hydroxypethidine, levallorphan, levorphanol, meperidine, mephentermine, mescaline, methadone, methamphetamine, methylenedioxyamphetamine, methylephedrine, methylphenidate, monoacetylmorphine, morphine, morphine-3-glucuronide, morphine-N-oxide, norcodeine, norlevorphanol, normetanephine, normethadone, normorphine, norpethidine, norpiperone, norpseudoephedrine, oxycodone, pentazocine, phenelzine, 2-phenethylamine, phentermine, phenylephrine, phenylpropanolamine, pholcodine, pipradol, prolintane, pseudoephedrine, STP, thebacon, thebaine, trimethoxyamphetamine, tyramine

Noninterfering: dopamine, levodopa, methyl dopa, methyl dopate, norepinephrine

Interfering: benzphetamine, buprenorphine, caffeine, dextromoramide, diethylpropion, etorphine, fenethyline, fentanyl, mazindol, nalorphine, naloxone, noscapine, papaverine, pemoline, phenazocine, phendimetrazine, phenoperidine, piritramide, tranlycypromine

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

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Kinney, C.D.; Kelly, J.G. Liquid chromatographic determination of paracetamol and dextropropoxyphene in plasma. *J.Chromatogr.*, **1987**, *419*, 433-437 [verapamil (IS); electrochemical detection; column temp 30; LOD 2 ng/mL; pharmacokinetics]

Lurie, I.S.; McGuinness, K. The quantitation of heroin and selected basic impurities via reversed phase HPLC. II. The analysis of adulterated samples. *J.Liq.Chromatogr.*, **1987**, *10*, 2189-2204 [UV detection; electrochemical detection; also acetaminophen, acetylcodeine, acetylmorphine, acetylprocaine, aminopyrene, amitriptyline, antipyrine, aspirin, barbital, benztropine, caffeine, cocaine, codeine, diamorphine, diazepam, diphenhydramine, dipyrene, ephedrine, ethylmorphine, lidocaine, meconin, methamphetamine, methapyrilene, methaqualone, monoacetylmorphine, morphine, nalorphine, niacinamide, noscapine, papaverine, phenacetin, phenmetrazine, phenobarbital, phenolphthalein, procaine, pyrilamine, quinidine, quinine, salicylamide, salicylic acid, secobarbital, strychnine, tartaric acid, tetracaine, thebaine, tripeleannamine, tropacocaine, vitamin B3, vitamin B5]

Schill, G.; Wainer, I.W.; Barkan, S.A. Chiral separation of cationic drugs on an α 1-acid glycoprotein bonded stationary phase. *J.Liq.Chromatogr.*, **1986**, *9*, 641-666 [chiral; also atropine, bromdiphenhydramine, brompheniramine, bupivacaine, butorphanol, carbinoxamine, chlorpheniramine, clidinium, cocaine, cyclopentolate, dimethindene, dipiperidone, disopyramide, doxylamine, ephedrine, homatropine, labetalol, labetalol A, labetalol B, mepensolate, mepivacaine, methadone, methorphan, methylatropine, methylhomatropine, methylphenidate, metoprolol, nadolol, nadolol A, nadolol B, oxprenolol, oxyphencyclimine, phenmetrazine, phenoxybenzamine, promethazine, pronethalol, propranolol, pseudoephedrine, terbutaline, tocinamide, tridihexethyl]

Wong, S.H.Y.; McHugh, S.L.; Dolan, J.; Cohen, K.A. Tricyclic antidepressant analysis by reversed-phase liquid chromatography using phenyl columns. *J.Liq.Chromatogr.*, **1986**, *9*, 2511-2538 [also acetaminophen, amitriptyline, amobarbital, amoxapine, barbital, chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, codeine, desipramine, desmethyldoxepin, diazepam, doxepin, fluphenazine, flurazepam, glutethimide, hydroxyamoxapine, imipramine, internal standard, lorazepam, maprotiline, meperidine, metabolites, nortriptyline, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, protriptyline, secobarbital, thioridazine, trazodone]

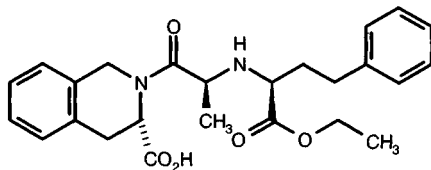
Angelo, H.R.; Kranz, T.; Strom, J.; Thisted, B.; Bredgaard, M. High-performance liquid chromatographic method for the determination of dextropropoxyphene and nordextropropoxyphene in serum. *J.Chromatogr.*, **1985**, *345*, 413-418 [pyrroliphen (IS); LOD 100 nM; simultaneous clomipramine, clopenthixol, codeine, ketobemidone, levomepromazine, meperidine, methadone; non-interfering acetaminophen, amitriptyline, aprobarbital, barbital, carbamazepine, chlordiazepoxide, chlorpromazine, chlorprothixene, desimipramine, diazepam, disopyramide, flupenthixol, imipramine, lidocaine, methadone, morphine, nitrazepam, nortriptyline, oxazepam, phenobarbital, phenytoin, theophylline, thioridazine]

Quinapril

Molecular formula: C₂₅H₃₀N₂O₅

Molecular weight: 438.5

CAS Registry No.: 85441-61-8 (quinapril),
90243-99-5 (quinapril hydrochloride monohydrate),
82586-55-8 (quinapril hydrochloride)



HCl

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 100 mg 1 mL Bond-Elut C18 SPE cartridge with two 1 mL portions of MeOH, two 1 mL portions of water, and two 1 mL portions of 100 mM HCl. Condition a CBA-Bond-Elut SPE cartridge with MeOH and water. 1 mL Plasma + 1 mL buffer + 250 ng IS (or 50-500 μ L urine + 500 ng IS), add to the C18 SPE cartridge, wash with two 1 mL portions of pH 3.4 water, wash with two 1 mL portions of distilled n-hexane, dry under vacuum for 20-30 min, elute with three 1 mL portions of chloroform:MeOH 2:1. Evaporate the eluate to dryness, reconstitute with 50 μ L chloroform:MeOH 50:50 and 50 μ L 2 mg/mL 9-anthryldiazomethane in MTBE, vortex for a few s, heat at 40° for 90 min, evaporate to dryness, reconstitute with two 100 μ L portions of MeCN, add to the CBA SPE cartridge, wash with two 1 mL portions of MeCN, elute with three 1 mL portions of MeCN:triethylamine 99.8:0.2, evaporate the eluate to dryness under reduced pressure, reconstitute with 200 μ L MeCN, inject a 20-50 μ L aliquot. (Prepare 9-anthryldiazomethane as follows. Stir 8.8 g 9-anthraldehyde and 8.5 g 80% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) 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

Column: 125 \times 4.6 5 μ m Spherisorb ODS II

Mobile phase: MeCN:MeOH:water 45:40:15 containing 0.24% ammonium perchlorate and 0.02% triethylamine

Flow rate: 1.6

Injection volume: 20-50

Detector: F ex 360 em 440

CHROMATOGRAM

Retention time: 3.8

Internal standard: [2S-[1[R*(R*)],2R*]-1-[2-[[1-carboxy-3-phenyl)propyl]amino]-1-oxo-propyl]-octahydro-1H-indole-2-carboxylic acid (PD 110021, Warner-Lambert) (16)

Limit of detection: 5 ng/mL (plasma)

Limit of quantitation: 20 ng/mL (plasma); 100 ng/mL (urine)

OTHER SUBSTANCES

Extracted: metabolites, quinaprilat

Simultaneous: enalapril, enalaprilat

KEY WORDS

plasma; derivatization; pharmacokinetics; SPE

REFERENCE

Hengy, H.; Most, M. Determination of the new ACE-inhibitor quinapril and its active metabolite quinaprilate in plasma and urine by high-performance liquid chromatography and pre-column labelling for fluorescent-detection. *J.Liq.Chromatogr.*, **1988**, *11*, 517-530

SAMPLE

Matrix: perfusate

Sample preparation: Add taurocholic acid to perfusate, filter, inject an aliquot.

HPLC VARIABLES

Column: 5 μm Ultrasphere C18

Mobile phase: MeOH:50 mM pH 7.4 sodium phosphate buffer 70:30

Detector: UV 220

CHROMATOGRAM

Retention time: 5.5

Internal standard: taurocholic acid (7.5)

REFERENCE

Hu, M.; Zheng, L.; Chen, J.; Liu, L.; Zhu, Y.; Dantzig, A.H.; Stratford, R.E., Jr. Mechanisms of transport of quinapril in Caco-2 cell monolayers: Comparison with cephalixin. *Pharm.Res.*, **1995**, *12*, 1120-1125

SAMPLE

Matrix: perfusate, urine

Sample preparation: 100 μL Urine or perfusate + 200 μL MeCN, vortex for 5 s, sonicate for 5 min, centrifuge at 11150 g for 5 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 mm long 5 μm Econosil C8

Column: 250 \times 4.6 5 μm Econosil C8

Mobile phase: MeCN:buffer 55:45 (Buffer was 0.01% triethylamine adjusted to pH 2.00 with phosphoric acid.)

Flow rate: 1

Injection volume: 100

Detector: UV 215; Radioactivity

CHROMATOGRAM

Retention time: 3.4

OTHER SUBSTANCES

Extracted: metabolites, quinaprilat

KEY WORDS

tritium labelled; dog; pharmacokinetics

REFERENCE

Kugler, A.R.; Olson, S.C.; Smith, D.E. Determination of quinapril and quinaprilat by high-performance liquid chromatography with radiochemical detection, coupled to liquid scintillation counting spectrometry. *J.Chromatogr.B*, **1995**, *666*, 360-367

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:** 22

OTHER SUBSTANCES**Simultaneous:** benzepril, captopril, cilazapril, enalapril, ramipril

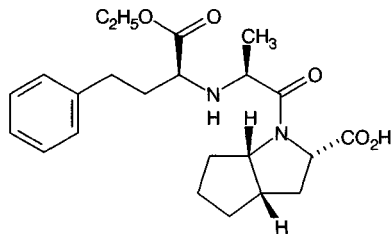
REFERENCEBarbato, F.; Morrica, P.; Quaglia, F. Analysis of ACE inhibitor drugs by high performance liquid chromatography. *Farmaco*, **1994**, *49*, 457–460

Ramipril

Molecular formula: C₂₃H₃₂N₂O₅

Molecular weight: 416.5

CAS Registry No.: 87333-19-5



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)) KH₂PO₄ 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: 6.04

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, acetaminophen, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazoxide, diclofenac, dihydralazine, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenpropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glipizide, glutethimide, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperridine, mephensesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nifedipine, niflumic acid, nimodipine, nitrazepam, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procabazine, proguanil, promethazine, propafenone, propran-

lolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ranitidine, ritalin, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocolmarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: aceprometazine, aconitine, alprenolol, bisoprolol, chlorophenacinone, diazepam, diltiazem, doxepin, glibenclamide, glibornuride, haloperidol, mianserine, nifedipine, nitrendipine, reserpine, tetracaine, vinblastine

KEY WORDS

whole blood; plasma

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: Water, apple juice. Centrifuge at 350 g for 10 min, filter (0.45 μm) the supernatant, dilute the filtrate with 1-2 volumes of water, inject a 50 μL aliquot. Applesauce. 1 g Applesauce + 5 mL 100 mM HCl, vortex for 2 min, centrifuge at 350 g for 10 min, filter (0.45 μm) the supernatant, dilute the filtrate with 1-2 volumes of water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: C18 (J.T. Baker)

Mobile phase: MeCN:buffer 38.5:61.5, adjusted to pH 2.1 ± 0.1 with concentrated phosphoric acid (Buffer was 14 g sodium perchlorate monohydrate and 100 mL 500 mM phosphoric acid made up to 1 L with water, pH adjusted to 2.5 ± 0.1 with triethylamine.)

Flow rate: 1

Injection volume: 50

Detector: UV 210

CHROMATOGRAM

Retention time: 12.1

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities, degradation products

KEY WORDS

water; apple juice; applesauce; stability-indicating

REFERENCE

Allen, L.V., Jr.; Stiles, M.L.; Prince, S.J.; McLaurry, H.-J.; Sylvestri, M.F. Stability of ramipril in water, apple juice, and applesauce. *Am. J. Health-Syst. Pharm.*, **1995**, *52*, 2433-2436

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 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: 16

OTHER SUBSTANCES

Simultaneous: benzepril, captopril, cilazapril, enalapril, quinapril

REFERENCE

Barbato, F.; Morrica, P.; Quaglia, F. Analysis of ACE inhibitor drugs by high performance liquid chromatography. *Farmaco*, **1994**, *49*, 457-460

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water:85% phosphoric acid 55:45:0.05
Detector: UV 215-220

REFERENCE

Ranadive, S.A.; Chen, A.X.; Serajuddin, A.T. Relative lipophilicities and structural-pharmacological considerations of various angiotensin-converting enzyme (ACE) inhibitors. *Pharm.Res.*, **1992**, *9*, 1480-1486

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 5 μm Nucleosil SA
Mobile phase: MeCN:buffer 2:1 (Buffer was 0.15% KH₂PO₄ adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Simultaneous: impurities, diastereomer (RS-SSS)

REFERENCE

Ito, M.; Kuriki, T.; Goto, J.; Nambara, T. Separation of ramipril optical isomers by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1990**, *13*, 991-1000

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Nucleosil 5 C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 0.15% KH₂PO₄ adjusted to pH 2.4 with phosphoric acid.)

Flow rate: 1

Detector: UV 210

CHROMATOGRAM

Retention time: k' 10.60

OTHER SUBSTANCES

Simultaneous: impurities, diastereomers (SS-RRR, RS-RRR, SR-SSS)

REFERENCE

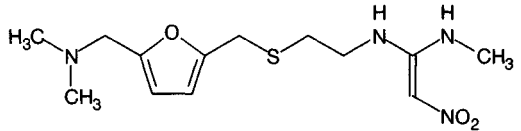
Ito, M.; Kuriki, T.; Goto, J.; Nambara, T. Separation of ramipril optical isomers by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1990**, *13*, 991–1000

Ranitidine

Molecular formula: C₁₃H₂₂N₄O₃S

Molecular weight: 314.4

CAS Registry No.: 66357-35-5, 66357-59-3 (HCl)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 25 μ L 10 μ g/mL procainamide in MeOH + 15 μ L 6 M NaOH + 2 mL ethyl acetate:isopropanol 96:4, shake mechanically for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μ L MeOH, inject a 75 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:10 mM pH 5.1 KH₂PO₄ 8:92

Flow rate: 2.5

Injection volume: 75

Detector: UV 330

CHROMATOGRAM

Retention time: 5

Internal standard: procainamide (3)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: lidocaine

Noninterfering: brompheniramine, chlorpheniramine, cimetidine, diazepam, diclofenac, glyburide, ibuprofen, ketoprofen, metoclopramide, naproxen, phenylbutazone, verapamil

KEY WORDS

plasma; pharmacokinetics

REFERENCE

al-Khamis, K.I.; El-Sayed, Y.M.; Al-Rashood, K.A.; Bawazir, S.A. High-performance liquid chromatographic determination of ranitidine in human plasma. *J.Liq.Chromatogr.*, **1995**, *18*, 277–286

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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 322

CHROMATOGRAM**Retention time:** 3.38**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amitriptyline, amodiaquine, amoxapine, astemizole, benazepril, benperidol, benzocaine, benzoylcegonine, bupridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cimetidine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenpropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenylbutazone, pimozone, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procabazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, reserpine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, suriclone, temazepam, tenoxicam, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: albuterol, amisulpride, aspirin, atenolol, chlormezanone, codeine, metformin, morphine, phenobarbital, phenol, ritodrine, sultopride, terbutaline, tiapride, toloxatone

KEY WORDS

whole blood; plasma

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: Mix plasma with sodium carbonate solution and extract twice with 3 mL dichloromethane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L mobile phase, inject a 40 μ L 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:** diclofenac

KEY WORDSplasma

REFERENCE

Van Gelderen, M.E.M.; Olling, M.; Barends, D.M.; Meulenbelt, J.; Salomons, P.; Rauws, A.G. The bio-availability 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:** Condition a 100 mg Bond Elut CN cartridge with 3 mL water, 3 mL MeOH, and 3 mL 50 mM pH 7.0 sodium phosphate buffer. Mix 4 volumes of plasma with 1 volume of 100 mM NaH₂PO₄. Add 0.5-1 mL plasma to the SPE cartridge, wash with 5 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness, reconstitute in 120 µL phosphate buffer, inject a 100 µL aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeOH:50 mM pH 7.0 sodium phosphate buffer 60:40**Flow rate:** 0.8**Injection volume:** 100**Detector:** UV 315

CHROMATOGRAM**Retention time:** 8**Limit of detection:** 1 ng/mL

KEY WORDSplasma; SPE; pharmacokinetics

REFERENCE

Biermann, B.; Sommer, N.; Winne, D.; Schumm, F.; Maier, U.; Breyer-Pfaff, U. Renal clearance of pyridostigmine in myasthenic patients and volunteers under the influence of ranitidine and pirenzepine. *Xenobiotica*, **1993**, *23*, 1263–1275

SAMPLE**Matrix:** blood**Sample preparation:** Condition a 100 mg LRC Bond Elut unendcapped cyanopropyl SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Serum or plasma + 250 µL 2 µg/mL procainamide in buffer, vortex, centrifuge at 1300 rpm for 10 min (plasma only), add 1 mL sample to the SPE cartridge, wash twice with 1 mL water, dry with nitrogen for 30 s, elute with two 250 µL aliquots of MeOH:buffer 60:40, inject 100 µL aliquot of eluate.

(Buffer was 50 mM KH_2PO_4 and Na_2HPO_4 50:50 v/v adjusted to pH 6.0 with phosphoric acid.)

HPLC VARIABLES

Guard column: 15 × 4.6 7 μm Brownlee RP-18

Column: 250 × 4.6 5 μm Spherisorb ODS-1

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 50 mM KH_2PO_4 and Na_2HPO_4 50:50 v/v adjusted to pH 6.0 with phosphoric acid.)

Column temperature: 48

Flow rate: 1.25

Injection volume: 100

Detector: UV 320

CHROMATOGRAM

Retention time: 4.5

Internal standard: procainamide (5.4)

Limit of quantitation: 10 ng/mL

KEY WORDS

serum; plasma; robotic sample preparation; SPE

REFERENCE

Lloyd, T.L.; Perschy, T.B.; Gooding, A.E.; Tomlinson, J.J. Robotic solid phase extraction and high performance liquid chromatographic analysis of ranitidine in serum or plasma. *Biomed.Chromatogr.*, **1992**, 6, 311-316

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 200 μL MeCN + 50 mg ZnSO_4 , vortex for 2 min, centrifuge at 2200 g for 3 min. Remove the supernatant, filter (0.45 μm), inject an aliquot. (Tubes were cleaned by sonication with acetone for 30 min, sonication twice with MeCN for 15 min, and drying at 100°. Stir 100 g ZnSO_4 crystals with 200 mL MeCN for 30 min, decant MeCN, repeat twice with fresh MeCN, dry crystals in a hood at room temperature.)

HPLC VARIABLES

Column: 50 × 4.1 Alltech PLRP-S

Mobile phase: MeCN:5 mM K_2HPO_4 + 0.5 mM tetraethylammonium hydroxide 20:80, pH 11

Flow rate: 1

Detector: UV 315

CHROMATOGRAM

Retention time: 7

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

Noninterfering: acetaminophen, ampicillin, cimetidine, creatinine, tetracycline

KEY WORDS

plasma

REFERENCE

Rustum, A.M. Rapid and sensitive HPLC determination of ranitidine in plasma. Application to pharmacokinetics study. *J.Liq.Chromatogr.*, **1988**, 11, 2315-2335

SAMPLE

Matrix: blood, milk

Sample preparation: 100 μ L Serum or whole milk + 25 μ L MeOH + 4 mL dichloromethane, vortex, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 RP-18 (Applied Biosystems)

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:water:glacial acetic acid:triethylamine 15:85:0.15:0.02

Flow rate: 1

Injection volume: 50

Detector: UV 228

CHROMATOGRAM

Retention time: 10.7

Internal standard: ranitidine

OTHER SUBSTANCES

Extracted: cimetidine

KEY WORDS

serum; whole milk; ranitidine is IS

REFERENCE

Oo, C.Y.; Kuhn, R.J.; Desai, N.; McNamara, P.J. Active transport of cimetidine into human milk. *Clin.Pharmacol.Ther.*, **1995**, *58*, 548-555

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 25 μ L Plasma + 100 μ L 75 μ M nizatidine + 100 μ L 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 1650 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot. (Hemolyze 25 μ L whole blood with 200 μ L water then proceed as above.) Tissue. Homogenize brain tissue with 100 μ L 30 μ M nizatidine and 1 mL saline at 0° for 1 min, add 100 μ L 500 mM NaOH, extract with 5 mL dichloromethane. Remove 3 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μ L mobile phase, centrifuge at 10000 g, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 Senshu gel 7C18H (Senshu, Tokyo)

Mobile phase: MeCN:buffer 5:95. (Buffer was 5 mM NaH₂PO₄ containing 5 mM tetramethylammonium chloride.)

Column temperature: 30

Flow rate: 2

Injection volume: 50

Detector: UV 320

CHROMATOGRAM

Internal standard: nizatidine

Limit of detection: 1 μ M (blood); 0.5 nmole/g (brain)

KEY WORDS

plasma; mouse; brain; whole blood

REFERENCE

Shimokawa, M.; Yamamoto, K.; Kawakami, J.; Sawada, Y.; Iga, T. Effect of renal or hepatic dysfunction on neurotoxic convulsion induced by ranitidine in mice. *Pharm.Res.*, **1994**, *11*, 1519–1523

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 250 μ L Plasma + 75 μ L 20 μ g/mL metoclopramide + 300 μ L 2 M NaOH, vortex for 30 s, add 5 mL dichloromethane, mix for 2 min, centrifuge at 10000 rpm for 15 min. Remove the organic layer and evaporate to dryness under nitrogen. Reconstitute the residue in 150 μ L mobile phase, inject a 50 μ L aliquot. Urine. Dilute 100 μ L urine + 75 μ L 2 mg/mL metoclopramide to 10 mL with water, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Spherisorb S 5 ODS II

Mobile phase: MeCN:MeOH:buffer 60:30:5, pH adjusted to 3.8 with glacial acetic acid (Buffer was 50 mM ammonium acetate containing 10 mM sodium octane sulfonate.)

Flow rate: 1.2

Injection volume: 50

Detector: UV 330

CHROMATOGRAM

Retention time: 5

Internal standard: metoclopramide (8)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, chlorodiazepam, chlorpromazine, desmethyldiazepam, diazepam, oxazepam, theobromine, theophylline

KEY WORDS

plasma

REFERENCE

Shiekh Salem, M.; Gharaibeh, A.M.; Alkaysi, H.N.; Badwan, A. High-performance liquid chromatographic analysis of ranitidine in plasma and urine. *J.Clin.Pharm.Ther.*, **1988**, *13*, 351–357

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 2 mL Plasma + 200 μ L 5 M NaOH, vortex for a few seconds, add 10 mL dichloromethane, vortex for 10 min, centrifuge at 1200 g for 10 min. Remove the organic layer and evaporate it to dryness under nitrogen at 40°. Reconstitute in 100 μ L water, inject a 20–50 μ L aliquot. Urine. Dilute 1:50 with water, vortex for a few seconds, centrifuge at 1200 g for 5 min, extract the supernatant as described above.

HPLC VARIABLES

Guard column: 50 \times 4.6 Spherisorb CN

Column: 150 \times 4.6 6 μ m Zorbax CN

Mobile phase: MeCN:50 mM KH₂PO₄ 15:85 containing 5 mM octanesulfonic acid

Flow rate: 2

Injection volume: 20–50

Detector: UV 318

CHROMATOGRAM

Retention time: 3.1

Internal standard: AH 20480 (4.2)

Limit of detection: 0.5 ng/mL

KEY WORDS

plasma

REFERENCE

Mullersman, G.; Derendorf, H. Rapid analysis of ranitidine in biological fluids and determination of its erythrocyte partitioning. *J.Chromatogr.*, **1986**, *381*, 385–391

SAMPLE

Matrix: formulations

Sample preparation: 100 μ L Injection + 50 μ L 200 μ g/mL caffeine in water + 850 μ L water, vortex briefly, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Ultrasphere-ODS C18

Mobile phase: MeCN:buffer 8:92 (Buffer was 10 mM KH_2PO_4 adjusted to pH 5.0 with phosphoric acid or NaOH.)

Flow rate: 2

Injection volume: 50

Detector: UV 262

CHROMATOGRAM

Retention time: 6

Internal standard: caffeine (3)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

stability-indicating; injections

REFERENCE

Hoyer, G.L.; LeDoux, J.; Nolan, P.E., Jr. A sensitive stability indicating assay for the H_2 blocker ranitidine. *J.Liq.Chromatogr.*, **1995**, *18*, 1239–1249

SAMPLE

Matrix: formulations

Sample preparation: Dilute 1:4, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Adsorbosphere C18

Mobile phase: MeOH:100 mM ammonium acetate 65:35

Flow rate: 1

Injection volume: 20

Detector: UV 322

CHROMATOGRAM

Retention time: 8.0

OTHER SUBSTANCES

Noninterfering: ondansetron, paclitaxel

KEY WORDS

injections; 5% dextrose; stability-indicating

REFERENCE

Burm, J.-P.; Jhee, S.S.; Chin, A.; Moon, Y.S.K.; Jeong, E.; Nii, L.; Fox, J.L.; Gill, M.A. Stability of paclitaxel with ondansetron hydrochloride or ranitidine hydrochloride during simulated Y-site administration. *Am.J.Hosp.Pharm.*, **1994**, *51*, 1201–1204

SAMPLE

Matrix: formulations

Sample preparation: Dilute 4:1 with saline, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Adsorbosphere

Mobile phase: MeOH:100 mM ammonium acetate 65:35, pH adjusted to 4.0 with 1 M NaOH

Flow rate: 1.2

Injection volume: 20

Detector: UV 322

CHROMATOGRAM

Retention time: 7.5

KEY WORDS

injections; saline; stability-indicating

REFERENCE

Choi, J.-S.; Burm, J.-P.; Jhee, S.S.; Chin, A.; Ulrich, R.W.; Gill, M.A. Stability of piperacillin sodium-tazobactam sodium and ranitidine hydrochloride in 0.9% sodium chloride injection during simulated Y-site administration. *Am.J.Hosp.Pharm.*, **1994**, *51*, 2273–2276

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Grind tablets to a fine powder, weigh out a quantity equivalent to about 150 mg ranitidine hydrochloride, add 50 mL MeOH:water 1:1, sonicate for 10 min, dilute to 100 mL with MeOH:water 1:1, filter, dilute 5 mL filtrate to 100 mL with MeOH:water 1:1, inject a 20 μ L aliquot. Injections, syrup. Measure out a volume equivalent to about 75 mg ranitidine hydrochloride, dilute to 50 mL with MeOH:water 1:1, mix, dilute 5 mL to 100 mL with MeOH:water 1:1, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Microsorb-MV C18

Mobile phase: MeOH:10 mM Na₂HPO₄ adjusted to pH 7.0 with phosphoric acid 50:50

Flow rate: 1

Injection volume: 20

Detector: UV 320

CHROMATOGRAM

Retention time: 3.44

KEY WORDS

tablets; injections; syrups

REFERENCE

Lau-Cam, C.A.; Rahman, M.; Roos, R.W. Rapid reversed phase high performance liquid chromatographic assay method for ranitidine hydrochloride in dosage forms. *J.Liq.Chromatogr.*, **1994**, *17*, 1089–1104

SAMPLE

Matrix: formulations

Sample preparation: 100 μ L Solution + 1.9 mL MeOH:water 20:80, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 5 μ m Adsorbosphere C18

Column: 250 \times 4.6 5 μ m Adsorbosphere C18

Mobile phase: MeOH:100 mM ammonium acetate 65:35

Flow rate: 1

Injection volume: 50

Detector: UV 322

CHROMATOGRAM

Retention time: 6.5

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: hepatocyte suspensions

Sample preparation: Add 2 volumes acetone to the hepatocyte suspension, centrifuge at 13000 g for 5 min. Remove the clear supernatant and evaporate it to dryness in a centrifugal evaporator, reconstitute the residue in 1 mL MeOH, sonicate, centrifuge at 13000 g for 5 min, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m ODS 1 (Jones Chromatography)

Mobile phase: MeCN:MeOH:35 mM pH 7.0 phosphate buffer 5:46:49

Column temperature: 40

Detector: UV 320

CHROMATOGRAM

Retention time: 6.2

Limit of quantitation: 10 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

dog; rat

REFERENCE

Cross, D.M.; Bell, J.A.; Wilson, K. Kinetics of ranitidine metabolism in dog and rat isolated hepatocytes. *Xenobiotica*, **1995**, *25*, 367-375

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

OTHER SUBSTANCES**Also analyzed:** atenolol, cimetidine, hydrochlorothiazide

KEY WORDS¹⁴C labeled

REFERENCE

Collett, A.; Sims, E.; Walker, D.; He, Y.-L.; Ayrton, J.; Rowland, M.; Warhurst, G. Comparison of HT29-18-C₁ and Caco-2 cell lines as models for studying intestinal paracellular drug absorption. *Pharm.Res.*, **1996**, *13*, 216–221

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' 0.96

OTHER SUBSTANCES**Also analyzed:** acebutolol, 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, pyrillamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelenamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan, R.; Nasal, A.; Turowski, M. Binding site for basic drugs on α₁-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: 5.88 (A), 3.27 (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, fenpropfen, fentanyl, flvoxate, 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, methyl dopa, 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, pimizide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimepazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, 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, pyrillamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 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, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phen-tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piri-tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldi-amine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thi-othixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, tra-zodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelethamine, 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 electro-chemical oxidation detection. *J.Chromatogr.*, **1985**, *323*, 191–225

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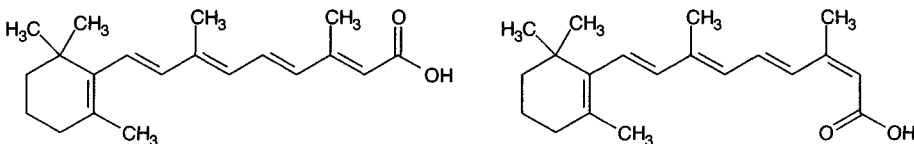
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- Lant, M.S.; Martin, L.E.; Oxford, J. Qualitative and quantitative analysis of ranitidine and its metabolites by high-performance liquid chromatography-mass spectrometry. *J.Chromatogr.*, **1985**, *323*, 143–152 [UV detection; LC-MS]
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Retinoic Acid

Molecular formula: C₂₀H₂₈O₂

Molecular weight: 300.4

CAS Registry No.: 302-79-4 (tretinoin (all-trans)), 4759-48-2 (isotretinoin (13-cis))



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 μ L Plasma + 20 μ L 1 μ g/mL acitretin in 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 μ L pentafluorobenzyl bromide and 10 μ L 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 μ L 10 mM BHT in MeCN, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 Nova-Pak C18 + 75 \times 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 299

CHROMATOGRAM

Retention time: 26 (isotretinoin), 28.2 (tretinoin)

Internal standard: acitretin (m/z 325) (16)

Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES

Extracted: 9-cis-retinoic acid

KEY WORDS

plasma; protect from light; derivatization; SPE

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

Sample preparation: 10 μL Serum + 30 μL 200-500 ng/mL retinyl acetate in isopropanol:dichloroethane 2:1 + 5 μL glacial acetic acid, vortex for 30 s, centrifuge for 1 min, inject a 10-20 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Upchurch)

Column: 150 \times 4.6 Ultracarb 5 ODS30 (Phenomenex)

Mobile phase: MeCN:dichloromethane:MeOH 85:12:3 containing 0.1% ammonium acetate (dissolve ammonium acetate in MeOH first)

Flow rate: 1

Injection volume: 10-20

Detector: UV 335

CHROMATOGRAM

Retention time: 9 (tretinoin)

Internal standard: retinyl acetate (5.5)

Limit of detection: 10 ng/mL

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: vitamin A

KEY WORDS

protect from light; serum

REFERENCE

Barua, A.B.; Kostic, D.; Barua, M.; Olson, J.A. Determination of retinol and retinoic acid in capillary blood by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1995**, *18*, 1459-1471

SAMPLE

Matrix: blood

Sample preparation: Condition a Bakerbond SPE octadecyl SPE cartridge with 2 mL MeOH and 2 mL 1 M acetic acid. 1 mL Serum + 3 mL 1 M acetic acid + 50 μL 10 μM IS in DMSO, mix, add to the SPE cartridge, wash with 2 mL acetone:1 M acetic acid 50:50, dry under vacuum for 15 min, elute with 500 μL MeCN. Evaporate the eluate and take up the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4 5 μm Lichrospher Si-60

Mobile phase: Hexane:dichloromethane:1,4-dioxane 78:18:4 containing 1% acetic acid

Flow rate: 0.8

Injection volume: 50

Detector: UV 360

CHROMATOGRAM

Retention time: 6 (isotretinoin), 7 (tretinoin)

Internal standard: (all-E)-3-methyl-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2,4,6-octanoic acid (Ro 13-6307) (7.5)

Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

protect from light; serum; SPE; normal phase; pharmacokinetics

REFERENCE

Lefebvre, P.; Agadir, A.; Cornic, C.; Gourmel, B.; Hue, B.; Dreux, C.; Degos, L.; Chomienne, C. Simultaneous determination of all-*trans* and 13-*cis* retinoic acids and their 4-oxo metabolites by adsorption liquid chromatography after solid-phase extraction. *J.Chromatogr.B*, **1995**, *666*, 55–61

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 1 mL IS in EtOH, vortex for 30 s, add 5 mL water, add 7.5 mL n-hexane, add 300 μ L 2 M HCl, rotate for 10 min, centrifuge at 1250 g for 8 min. Remove the organic layer and evaporate it at room temperature under a stream of nitrogen. Dissolve the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Spherisorb S5W

Mobile phase: n-Hexane:isopropanol:acetic acid 200:0.7:0.135

Flow rate: 0.9

Injection volume: 50

Detector: UV 350

CHROMATOGRAM

Retention time: 8 (isotretinoin), 11 (tretinoin)

Internal standard: Ro 15-1570 (22)

Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: retinol

KEY WORDS

plasma; normal phase

REFERENCE

Meyer, E.; Lambert, W.E.; De Leenheer, A.P. Simultaneous determination of endogenous retinoic acid isomers and retinol in human plasma by isocratic normal-phase HPLC with ultraviolet detection. *Clin.Chem.*, **1994**, *40*, 48–51

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 2 mL diluting agent (amber tube), mix for 10 s, add 5 mL n-hexane, vortex for 30 s, centrifuge at 1600 g at 4° for 5 min. Remove the organic phase and concentrate to < 1 mL under vacuum below 30°, evaporate the rest of the solvent under nitrogen, dissolve the residue in 25 μ L MeCN:MeOH 2:1, mix for 30 s, centrifuge at 8000 g for 1 min, inject a 20 μ L aliquot. (Diluting agent was MeCN:100 mM ammonium acetate 25:75, pH adjusted to 5.5 with acetic acid.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Chemcosorb 5-ODS-H

Mobile phase: MeCN:MeOH:100 mM ammonium acetate 46.7:23.3:30, pH adjusted to 7.0

Column temperature: 50

Flow rate: 1

Injection volume: 20

Detector: UV 340

CHROMATOGRAM**Retention time:** 14.9 (isotretinoin), 17.0 (tretinoin)**Limit of quantitation:** 0.5 ng/mL

OTHER SUBSTANCES**Extracted:** vitamin A (retinol)

KEY WORDSserum

REFERENCE

Takeda, N.; Yamamoto, A. Simultaneous determination of 13-cis- and all-trans-retinoic acids and retinol in human serum by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *657*, 53–59

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Plasma or serum + 100 μ L 6 μ g/mL 9-methylanthracene in MeOH + 1.5 mL MeCN + 100 μ L 100 mM perchloric acid, flush headspace of vial with argon, vortex, centrifuge, inject a 50 μ L aliquot of the supernatant. Sonicate serum, solvents, and mobile phase under vacuum before use. Use low-actinic glassware and yellow light.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Zorbax C18**Mobile phase:** MeCN:0.5% acetic acid 85:15 containing 0.05% sodium hexanesulfonate**Flow rate:** 2**Injection volume:** 50**Detector:** UV 365

CHROMATOGRAM**Retention time:** 6 (isotretinoin), 8 (tretinoin)**Internal standard:** 9-methylanthracene (5)**Limit of detection:** 12 ng/mL

OTHER SUBSTANCES**Simultaneous:** 4-oxo-13-cis-retinoic acid, retinol

KEY WORDSplasma; serum

REFERENCE

Gadde, R.R.; Burton, F.W. Simple reversed-phase high-performance liquid chromatographic method for 13-cis-retinoic acid in serum. *J.Chromatogr.*, **1992**, *593*, 41–46

SAMPLE**Matrix:** blood**Sample preparation:** 0.5-2 mL Plasma + 100 μ L 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 μ L MeOH, inject a 25 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m 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 (isotretinoin), 15 (tretinoin)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: acitretin, 13-cis-acitretin, etretinate, 4-oxo-13-cis-retinoic acid

Noninterfering: antidepressants, benzodiazepines, psoralen

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 μ L Plasma + 750 μ L 100 ng/mL acitretin in MeCN:9 mM NaOH 20:80, centrifuge at 1500 g for 3 min, inject a 500 μ L 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 10° in the autosampler.)

HPLC VARIABLES

Column: A 14 \times 4.6 37-50 μ m Bondapak C18 Corasil (column fitted with 3 μ m sieves not glass fiber filters); B 30 \times 4 5 μ m Spherisorb ODS 1 + 125 \times 4 5 μ m Spherisorb ODS 1 + 125 \times 4 5 μ m 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 70:30 over 6 min, then to 0:100 over 5 min, stay at 0:100 for 11 min.

Flow rate: A 1.5; B 1

Injection volume: 500

Detector: UV 360

CHROMATOGRAM

Retention time: 25 (isotretinoin), 27 (tretinoin)

Internal standard: acitretin (23)

Limit of detection: 0.5-1 ng/mL

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, isotretinoin, 4-oxoisotretinoin, 4-oxotretinoin

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 μ L Plasma + 50 μ L 5% perchloric acid, vortex for 30 s, add 500 μ L ethyl acetate, whirl for 1 min, centrifuge at 13000 g for 1 min, inject a 50 μ L aliquot of the organic layer.

HPLC VARIABLES**Guard column:** present but not specified**Column:** 250 \times 4.6 5 μ m Ultrasphere ODS + 300 \times 4 10 μ m μ Bondapak in series**Mobile phase:** MeCN:1% ammonium acetate 95:5**Flow rate:** 2.5**Injection volume:** 50**Detector:** UV 340; UV 365

CHROMATOGRAM**Retention time:** 6.5 (isotretinoin), 8.2 (tretinoin)

OTHER SUBSTANCES**Extracted:** vitamin A

KEY WORDS

protect from light; plasma

REFERENCEPeng, Y.-M.; Xu, M.-J.; Alberts, D.S. Analysis and stability of retinol in plasma. *J.Natl.Cancer Inst.*, 1987, 78, 95-99

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Plasma + 100 μ L 5% perchloric acid, mix rapidly, add 500 μ L ethyl acetate, mix for 60-90 s, centrifuge at 13000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES**Column:** Reversed-phase C18 (Waters or Bio-Rad)**Mobile phase:** MeCN:1% ammonium acetate 75:25**Flow rate:** 2.5**Detector:** UV 340

CHROMATOGRAM**Retention time:** 3 (isotretinoin), 4 (tretinoin)**Limit of detection:** 20 ng/mL

OTHER SUBSTANCES**Extracted:** vitamin A

KEY WORDS

plasma; pharmacokinetics; protect from light

REFERENCEDavis, 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, microsomal incubations

Sample preparation: 500 μL Plasma or 250 μL microsomal incubation + 25 μL 20 $\mu\text{g}/\text{mL}$ IS in MeCN + 350 μL 1-butanol:MeCN 50:50, mix thoroughly, add 300 μL saturated K_2HPO_4 , mix, centrifuge at 3000 g for 10 min, inject an aliquot of the organic layer.

HPLC VARIABLES

Column: Adsorbosphere C18

Mobile phase: Gradient. MeCN:10 mM ammonium acetate from 50:50 to 95:5 over 10 min

Flow rate: 1.5

Detector: UV 365

CHROMATOGRAM

Internal standard: Ro 23-4736

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; protect from light; human; liver; for tretinoin; pharmacokinetics

REFERENCE

Schwartz, E.L.; Hallam, S.; Gallagher, R.E.; Wiernik, P.H. Inhibition of all-*trans*-retinoic acid metabolism by fluconazole *in vitro* and in patients with acute promyelocytic leukemia. *Biochem.Pharmacol.*, 1995, 50, 923-928

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Add 5 volumes of MeOH to plasma, cool to -20° , centrifuge at 16500 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in mobile phase, centrifuge at 16500 g for 5 min, inject a 10-250 μL aliquot. Liver. Homogenize (Kinematica-Polytron with an Aggregat PTA 10TS generator) rat liver with 5 volumes of MeOH at 20000 rpm for 20 s, let stand at -20° overnight, centrifuge at $0-4^\circ$ at 3200 g for 10 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in mobile phase, centrifuge at 16500 g for 5 min, inject a 10-250 μL aliquot.

HPLC VARIABLES

Guard column: $20 \times 4.5 \mu\text{m}$ C18 (Hewlett-Packard or Alltech)

Column: $250 \times 4.6 \mu\text{m}$ Microsorb-MV

Mobile phase: MeCN:10 mM ammonium acetate:glacial acetic acid 80:20:1

Column temperature: 40

Flow rate: 1

Injection volume: 10-250

Detector: UV 348

CHROMATOGRAM

Retention time: 13.6 (isotretinoin), 16.3 (tretinoin)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; plasma; liver

REFERENCE

Shirley, M.A.; Bennani, Y.L.; Boehm, M.F.; Breau, A.P.; Pathirana, C.; Ulm, E.H. Oxidative and reductive metabolism of 9-*cis*-retinoic acid in the rat. Identification of the 13,14-dihydro-9-*cis*-retinoic acid and its taurine conjugate. *Drug Metab.Dispos.*, **1996**, *24*, 293–302

SAMPLE

Matrix: culture media

Sample preparation: 100 μ L Culture media + 200 μ L 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 \times 8 5 μ m 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: 9.44 (tretinoin)

OTHER SUBSTANCES

Extracted: acitretin, etretinate, isotretin, motretinid, retinal, vitamin A

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: solutions

Sample preparation: Inject an aliquot of a solution in the mobile phase.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Zorbax Rx-Sil

Mobile phase: Heptane:THF:acetic acid 96.5:3.5:0.015

Flow rate: 1.4

Detector: MS, Finnigan 4023, particle beam interface (Vestec universal interface model 700), electron impact mode 77 eV, scan *m/z* 200-350 (positive ion), multiplier voltage 1200 V, source 300°; UV 365

CHROMATOGRAM

Retention time: 13 (isotretinoin), 16 (tretinoin)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

normal phase

REFERENCE

Bempong, D.K.; Honigberg, I.L.; Meltzer, N.M. Normal phase LC-MS determination of retinoic acid degradation products. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 285–291

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)

Mobile phase: MeOH:water 92:8

Flow rate: 1

Detector: UV 330

CHROMATOGRAM

Retention time: 3.5 (tretinoin)

OTHER SUBSTANCES

Simultaneous: retinol acetate, vitamin A, vitamin E

REFERENCE

Jedrejewski, P.T.; Taylor, L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography. *J.Chromatogr.Sci.*, **1995**, *33*, 438–445

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Suplex-pKb-100 (Supelco)

Mobile phase: MeOH:acetic acid 99.95:0.05

Flow rate: 1

Detector: UV 350

CHROMATOGRAM

Retention time: 13.5 (isotretinoin), 22 (tretinoin)

OTHER SUBSTANCES

Simultaneous: other isomers

KEY WORDS

photoisomerization solutions

REFERENCE

Sundquist, A.R.; Stahl, W.; Steigel, A.; Sies, H. Separation of retinoic acid all-trans, mono-cis and poly-cis isomers by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *637*, 201–205

SAMPLE

Matrix: tissue

Sample preparation: 100-120 mg Frog embryos + 100 μL isopropanol, sonicate on ice, vortex for 1 min, centrifuge at 4° at 4000 g for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4 10 μm LiChrosorb RB 18

Column: 125 × 4.6 3 μm Spherisorb ODS II

Mobile phase: Gradient. MeOH:40 mM pH 7.3 ammonium acetate from 55:45 to 100:0 over 18 min.

Flow rate: 1.6

Detector: UV 354

CHROMATOGRAM

Retention time: 12.5 (isotretinoin), 12.7 (9-cis-retinoic acid), 13.5 (tretinoin)

OTHER SUBSTANCES

Extracted: metabolites, vitamin A

KEY WORDS

frog; embryo

REFERENCE

Creech Kraft, J.; Juchau, M.R. *Xenopus laevis*: A model system for the study of embryonic retinoid metabolism. III. Isomerization and metabolism of all-*trans*-retinoic acid and 9-*cis*-retinoic acid and their dysmorphogenic effects in embryos during neurulation. *Drug Metab.Dispos.*, **1995**, *23*, 1058–1071

SAMPLE

Matrix: tissue

Sample preparation: 100-120 mg Tadpole embryos + 100 mL isopropanol, sonicate on ice, vortex for 1 min, centrifuge at 4000 g at 4° for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4 10 μm LiChrosorb RB 18

Column: 125 × 4.6 3 μm Spherisorb ODS II

Mobile phase: Gradient. MeOH:40 mM pH 7.3 ammonium acetate from 55:45 to 100:0 over 20 min

Flow rate: 1.6

Detector: UV 354

CHROMATOGRAM

Retention time: 11.8 (isotretinoin), 12.2 (tretinoin)

OTHER SUBSTANCES

Extracted: metabolites, tretinoin, vitamin A

KEY WORDS

handle under yellow light; tadpoles; embryos

REFERENCE

Creech Kraft, J.; Kimelman, D.; Juchau, M.R. *Xenopus Laevis*: A model system for the study of embryonic retinoid metabolism. I. Embryonic metabolism of 9-*cis*- and all-*trans*-retinals and retinols and their corresponding acid forms. *Drug Metab.Dispos.*, **1995**, *23*, 72–82

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Achkar, C.C.; Bentel, J.M.; Boylan, J.F.; Scher, H.I.; Gudas, L.J.; Miller, W.H., Jr. Differences in the pharmacokinetic properties of orally administered all-*trans*-retinoic acid and 9-*cis*-retinoic acid in the plasma of nude mice. *Drug Metab.Dispos.*, **1994**, *22*, 451–458 [gradient; LOD 0.5 ng/mL; extracted metabolites, isotretinoin, tretinoin, vitamin A]

Eckhoff, C.; Chari, S.; Kromka, M.; Staudner, H.; Juhasz, L.; Rudiger, H.; Agnish, N. Teratogenicity and transplacental pharmacokinetics of 13-*cis*-retinoic acid in rabbits. *Toxicol.Appl.Pharmacol.*, **1994**, *125*, 34–41 [plasma; acitretin (IS); SPE; column temp 35; tissue; extracted metabolites, isotretinoin, tretinoin]

Guiso, G.; Rambaldi, A.; Dimitrova, B.; Biondi, A.; Caccia, S. Determination of orally administered all-*trans*-retinoic acid in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *656*, 239–244 [all-*trans*-retinyl acetate (IS); extracted metabolites, isotretinoin, tretinoin; LOD 10 ng/mL; pharmacokinetics; non-interfering allopurinol, amikacin, aracytin, ceftazidime, ciprofloxacin, doxorubicin, fluconazole, prednisone]

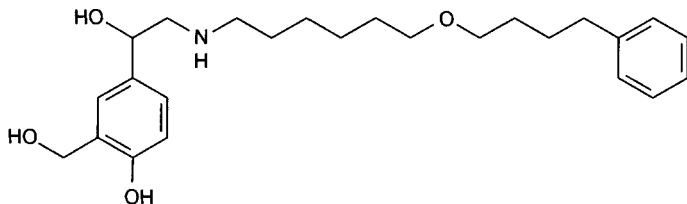
- Jiang, X.G.; Xi, N.Z. [A reversed-phase HPLC method for determining tretinoin]. *Chung Kuo Yao Li Hsueh Pao*, **1994**, *15*, 458–461
- Sass, J.O.; Nau, H. Single-run analysis of isomers of retinoyl- β -D-glucuronide and retinoic acid by reversed-phase high-performance liquid chromatography. *J.Chromatogr.A*, **1994**, *685*, 182–188 [LOD 0.25 ng; column temp 60; gradient; simultaneous metabolites, isotretinoin, tretinoin]
- Ranelder, U.B.; Lausecker, B.B.; Huselton, C. Micro liquid chromatography-mass spectrometry with direct liquid introduction used for separation and quantitation of all-*trans*- and 13-*cis*-retinoic acids and their 4-oxo metabolites in human plasma. *J.Chromatogr.*, **1993**, *617*, 129–135 [LC-MS; LOQ 0.3 ng/mL; extracted metabolites, isotretinoin, tretinoin]
- Tan, X.; Meltzer, N.; Lindenbaum, S. Solid-state stability studies of 13-*cis*-retinoic acid and all-*trans*-retinoic acid using microcalorimetry and HPLC analysis. *Pharm.Res.*, **1992**, *9*, 1203–1208 [solutions; simultaneous degradation products, isotretinoin, tretinoin]
- Bryan, P.D.; Honigberg, I.L.; Meltzer, N.M. Electrochemical detection of retinoids using normal phase HPLC. *J.Liq.Chromatogr.*, **1991**, *14*, 2287–2295 [LOD 1 ng; also acitretin, isotretinoin, tretinoin, vitamin A palmitate]
- Dobie, A.K.; Yang, K.Y.; De, N.C. High-performance liquid chromatographic procedure for retinoic acid in ophthalmic solution. *J.Liq.Chromatogr.*, **1991**, *14*, 1219–1226
- Creech Kraft, J.; Echoff, C.; Kuhnz, W.; Löfberg, B.; Nau, H. Automated determination of 13-*cis*- and all-*trans*-retinoic acid, their 4-oxo- metabolites and retinol in plasma, amniotic fluid and embryo by reversed-phase high-performance liquid chromatography with a precolumn switching technique. *J.Liq.Chromatogr.*, **1988**, *11*, 2051–2069 [gradient; LOD 2 ng/mL; mouse; extracted metabolites, isotretinoin, tretinoin, vitamin A]
- Wyss, R.; Bucheli, F. Quantitative analysis of retinoids in biological fluids by high-performance liquid chromatography using column switching. I. Determination of isotretinoin and tretinoin and their 4-oxo metabolites in plasma. *J.Chromatogr.*, **1988**, *424*, 303–314 [plasma; LOQ 2 ng/mL; extracted metabolites, isotretinoin, tretinoin; etretin (IS)]
- Furr, H.C.; Amédée-Manesme, O.; Olson, J.A. Gradient reversed-phase high-performance liquid chromatographic separation of naturally occurring retinoids. *J.Chromatogr.*, **1984**, *309*, 299–307 [gradient; rat; human; pig; liver; kidney; extracted retinyl esters, tretinoin, vitamin A]
- Shelley, R.; Price, J.C.; Jun, H.W.; Cadwallader, D.E.; Capomacchia, A.C. Improved and rapid high-performance liquid chromatographic assay for 13-*cis*-retinoic acid or all-*trans*-retinoic acid. *J.Pharm.Sci.*, **1982**, *71*, 262–264 [rat; serum; extracted isotretinoin, retinol acetate, tretinoin, vitamin A; pharmacokinetics; LOQ 100 ng/mL]
- Vane, F.M.; Stoltenborg, J.K.; Buggé, C.J. Determination of 13-*cis*-retinoic acid and its major metabolite, 4-oxo-13-*cis*-retinoic acid, in human blood by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1982**, *227*, 471–484 [gradient; whole blood; extracted metabolites, isotretinoin, tretinoin, vitamin A; LOQ 10 ng/mL; pharmacokinetics]

Salmeterol

Molecular formula: $C_{25}H_{37}NO_4$

Molecular weight: 415.6

CAS Registry No.: 89365-50-4



SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg C2 SPE cartridge with 1 mL MeOH and two 1 mL aliquots of water, do not allow to dry. Add 1 mL plasma to the SPE cartridge, wash with 1 mL water, allow to dry, wash with 1 mL MeCN, allow to dry, elute with 1.5 mL isopropanol. Evaporate the eluate to dryness under reduced pressure at 45°, reconstitute the residue in 100 μ L mobile phase, sonicate for 5 min, vortex, inject a 75 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m PLRP-S poly(styrene-divinylbenzene) (Polymer Laboratories)

Mobile phase: MeCN:MeOH:water:1 M ammonium sulfate 5:60:30:5, adjusted to pH 4.0 with sulfuric acid

Column temperature: 40

Flow rate: 1

Injection volume: 75

Detector: F ex 230 em 305

CHROMATOGRAM

Retention time: 7-8

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; dog; plasma; SPE; pharmacokinetics

REFERENCE

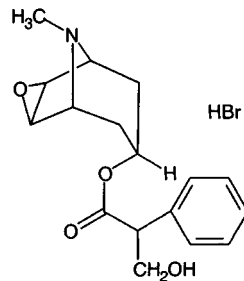
Colthup, P.V.; Young, G.C.; Felgate, C.C. Determination of salmeterol in rat and dog plasma by high-performance liquid chromatography with fluorescence detection. *J.Pharm.Sci.*, **1993**, *82*, 323-325

Scopolamine

Molecular formula: C₁₇H₂₁NO₄

Molecular weight: 303.4

CAS Registry No.: 51-34-3 (scopolamine), 6533-68-2 (scopolamine hydrobromide trihydrate), 114-49-8 (scopolamine hydrobromide anhydrous)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L MeOH, vortex briefly, add 50 μ L 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 2.5 μ m BDS C18 (Keystone)

Column: 50 \times 3.3 μ m 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: 0.8

Internal standard: scopolamine

OTHER SUBSTANCES

Extracted: hyoscyamine

KEY WORDS

plasma; protect from light; scopolamine is IS

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: 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 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 μ m 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: 10

OTHER SUBSTANCES

Simultaneous: hyoscyamine

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.*, **1989**, *37*, 2456–2458

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Crush tablet, add 2 mL water, add 500 μ L 0.1 M NaOH, if not basic to litmus add 0.1 M NaOH dropwise until it is. Extract with 10 mL chloroform then with three 5 mL portions of chloroform. Pass all extracts through a sodium sulfate column and then wash column with 2 mL chloroform. Evaporate all organic extracts with heating under a stream of air. Take up residue in 1 mL MeOH, inject a 100 μ L aliquot. Injections. Evaporate 2 mL to dryness on a steam bath, take up residue in 5 mL water, add 500 μ L 0.1 M NaOH, if not basic to litmus add 0.1 M NaOH dropwise until it is. Extract with 10 mL chloroform then with three 5 mL portions of chloroform. Pass all extracts through a sodium sulfate column and then wash column with 2 mL chloroform. Evaporate all organic extracts with heating under a stream of air. Take up residue in 2 mL MeOH, inject a 100 μ L aliquot. (Sodium sulfate column was a 300 \times 20 glass chromatography column containing 10 g anhydrous sodium sulfate washed with 10 mL chloroform before use.)

HPLC VARIABLES

Column: 250 \times 4.6 Alltech C18

Mobile phase: MeOH:20 g/L sodium pentanesulfonate 95:5

Flow rate: 1

Injection volume: 100

Detector: F ex 255 em 285

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: atropine

Noninterfering: ergotamine, phenobarbital

KEY WORDS

tablets; injections

REFERENCE

Cieri, U.R. Determination of small quantities of atropine in commercial preparations by liquid chromatography with fluorescence detection. *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 1042–1045

SAMPLE

Matrix: formulations

Sample preparation: Tablets, capsules. Powder tablets or remove contents of capsules, weigh out amount equivalent to about 600 μ g 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 $\mu\text{g}/\text{mL}$ theophylline in dichloromethane, distill 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 $\mu\text{g}/\text{mL}$ theophylline in dichloromethane, distill 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 \times 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: 5

Internal standard: theophylline (6.5)

OTHER SUBSTANCES

Simultaneous: atropine, hyoscyamine, phenobarbital

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: 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 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: 5.35

Limit of detection: 50 µg/g

OTHER SUBSTANCES

Simultaneous: hyoscyamine, tropic acid

KEY WORDS

freeze-dried plant leaves; leaves

REFERENCE

Fliniaux, M.-A.; Manceau, F.; Jacquín-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: 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: 9.3

OTHER SUBSTANCES

Simultaneous: hyoscyamine

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: 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 30:70

Flow rate: 1

Injection volume: 20

Detector: MS thermospray, VG Trio-2, ion source 150°, vaporizer tip 170°, repeller electrode 150 V, m/z 304

CHROMATOGRAM

Internal standard: homatropine (m/z 276)

Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: hyoscyamine

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: solutions

HPLC VARIABLES

Column: 150 × 2 PRP-1 (Keystone)

Mobile phase: MeCN:buffer 34:66 (Buffer was 80 mM phosphate and 20 mM borate, pH 9.0.)

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 pumped at 0.3 mL/min 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

KEY WORDS

post-column reaction

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

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: 6.95 (A), 3.70 (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, hydroalazine, 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, methyl dopa, 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, secobarbital, sertraline, sotalol, spironolactone, sulfipyrazone, 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

some 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: Centrifuge.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb S.50 DS1

Mobile phase: MeCN:buffer 50:50 (Buffer was 10 mM KH_2PO_4 + 0.0028% heptanesulfonic acid, pH 2.0.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 274

REFERENCE

Calpena, A.C.; Blanes, C.; Moreno, J.; Obach, R.; Domenech, J. A comparative in vitro study of transdermal absorption of antiemetics. *J.Pharm.Sci.*, **1994**, *83*, 29–33

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, dapson, 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, fenpropfen, 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, isocarboxystyryl, 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, methylidopa, methylidopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxypenbutazone, oxytetracycline, papaverine, pargyline, paxipine, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, praze-

pam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyriline, pyrilamine, pyrihydione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 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: 31 (d-isomer), 34 (l-isomer)

OTHER SUBSTANCES

Simultaneous: atropine, cocaine

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

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 $\mu\text{g/mL}$, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μm μ Bondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83

Flow rate: 1.5

Injection volume: 10

Detector: UV 230

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: atropine, atropine methyl, homatropine, methscopolamine, tropic acid

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

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Theodoridis, G.; Papadoyannis, I.; Vasilikiotis, G.; Tsoukali-Papadopoulou, H. Reversed-phase high-performance liquid chromatography–photodiode-array analysis of alkaloid drugs of forensic interest. *J.Chromatogr.B*, **1995**, *668*, 253–263 [also amphetamine, bamifylline, caffeine, cocaine, codeine, diamorphine, ethylmorphine, flufenamic acid, hyoscyamine, methadone, morphine, nalorphine, norcodeine, papaverine, quinine, strychnine, theobromine, theophylline, tolfenamic acid]

Whelpton, R.; Hurst, P.R.; Metcalfe, R.F.; Saunders, S.A. Liquid chromatographic determination of hyoscine (scopolamine) in urine using solid phase extraction. *Biomed.Chromatogr.*, **1992**, *6*, 198–204

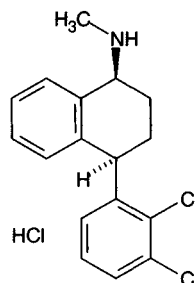
Achari, R.G.; Jacob, J.T. A study of the retention behavior of some basic drug substances by ion-pair HPLC. *J.Liq.Chromatogr.*, **1980**, *3*, 81–92 [also N-acetylprocainamide, antazoline, atropine, caffeine, chlorpheniramine, codeine, ephedrine, epinephrine, naphazoline, papaverine, pheniramine, phenylephrine, phenylpropanolamine, procainamide, quinidine, xylocaine]

Sertraline

Molecular formula: C₁₇H₁₇Cl₂N

Molecular weight: 306.2

CAS Registry No.: 79617-96-2 (sertraline), 79559-97-0 (sertraline hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 1 M HCl, twice with MeOH, and once with water. 500 μ L Serum + 200 μ L 500 ng/mL IS in 10 mg/mL potassium bicarbonate + 500 μ L MeCN, centrifuge, add to the SPE cartridge, wash twice with water, wash once with MeCN, elute (by gravity) with 250 μ L MeOH containing 25 mL/L 35% perchloric acid, inject a 20 μ L aliquot of the eluate.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:water:7% perchloric acid 400:750:0.5 containing 0.5 g tetramethylammonium perchlorate

Flow rate: 1.8

Injection volume: 20

Detector: UV 205

CHROMATOGRAM

Retention time: 11

Internal standard: CP-53,630-1 (8)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: desmethylsertraline

KEY WORDS

serum; SPE

REFERENCE

Gupta, R.N.; Dziurdzy, S.A. Therapeutic monitoring of sertraline. *Clin.Chem.*, **1994**, *40*, 498-499

SAMPLE

Matrix: blood

Sample preparation: 1 mL Blood + 100 μ L 1 μ g/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 μ L chloroform, extract. Remove the chloroform layer, evaporate to dryness under air at 60°, reconstitute in 100 μ L MeOH, inject a 20 μ L 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)

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: desmethylsertraline, diphenhydramine

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: Condition a 200 mg 10 mL Clean Screen copolymeric ZSDAU020 SPE cartridge with 3 mL MeOH, 3 mL water, and 1 mL 100 mM pH 6 KH_2PO_4 . 1 mL Serum + 2 mL 100 mM pH 6 KH_2PO_4 + 250 μL 2 $\mu\text{g}/\text{mL}$ protriptyline, add to the SPE cartridge, wash with 1 mL water, wash with 1 mL 1 M acetic acid, wash with 3 mL MeOH, dry under vacuum for 5 min, elute with 3 mL dichloromethane:isopropanol: ammonium hydroxide 78:20:2. Add a drop of diethylamine to the eluate and evaporate it to dryness at 40° under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μm Supelco LC-8

Mobile phase: MeCN:MeOH:water:diethylamine 1000:800:1200:2, containing 1.5 g sodium pentanesulfonate, pH adjusted to 5.5-6.0 with phosphoric acid

Flow rate: 1.5

Injection volume: 50

Detector: UV 600 for 2 min then UV 214

CHROMATOGRAM

Retention time: 9

Internal standard: protriptyline (6)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: desmethylsertraline

KEY WORDS

serum; SPE

REFERENCE

Rogowsky, D.; Marr, M.; Long, G.; Moore, C. Determination of sertraline and desmethylsertraline in human serum using copolymeric bonded-phase extraction, liquid chromatography and gas chromatography-mass spectrometry. *J.Chromatogr.B*, **1994**, *655*, 138–141

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 $\mu\text{g}/\text{mL}$ 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 μ L 1 M pH 10.3 carbonate buffer and 25 μ L 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 μ L MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-18

Mobile phase: MeCN:25 mM KH₂PO₄ 75:25 containing 500 μ L/L orthophosphoric acid and 600 μ L/L n-butylamine

Flow rate: 2

Injection volume: 25-40

Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM

Retention time: 25.60

Internal standard: maprotiline (12.8)

OTHER SUBSTANCES

Simultaneous: amoxapine, clovoxamine, desipramine, fenfluramine, fluoxetine, fluvoxamine, norfluoxetine, nortriptyline, propranolol, protriptyline

Noninterfering: amitriptyline, atenolol, bupropion, carbamazepine, chlordiazepoxide, citalopram, clomipramine, clozapine, cyclobenzaprine, doxepin, imipramine, loxapine, metoprolol, mianserin, moclobemide, nomifensine, pindolol, thioridazine, tranlycypromine, trazodone, trimipramine

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: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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: 14.50 (A), 7.68 (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, cimeti-

dine, 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, methyl dopa, 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, sotalol, spironolactone, sulfipyrazone, 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

some 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: Homogenize 1 mL liver in 5 mL water. Centrifuge 1 mL homogenate, add the supernatant to 1.2 µg clomipramine, add 75 µL MeOH, add 75 µL MeCN, add 100 µL 1 M HCl, vortex, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18

Mobile phase: MeCN:50 mM potassium phosphate buffer 35:65

Flow rate: 1.3

Detector: UV 213

CHROMATOGRAM

Internal standard: clomipramine

OTHER SUBSTANCES

Extracted: metabolites, desmethylsertraline

Also analyzed: fluoxetine

KEY WORDS

mouse; liver

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: tissue

Sample preparation: 130 mg Brain tissue + 1.1 mL EtOH + 3.3 µg tetracaine hydrochloride, disrupt by sonication, centrifuge, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.1 10 µm Versapack C18

Mobile phase: MeCN:250 mM pH 2.7 potassium phosphate buffer 30:70

Flow rate: 2

Injection volume: 20

Detector: UV 235

CHROMATOGRAM

Retention time: 12

Internal standard: tetracaine hydrochloride (4.9)

Limit of detection: 6.12 µg/g

OTHER SUBSTANCES

Extracted: desmethylsertraline

KEY WORDS

brain; mouse

REFERENCE

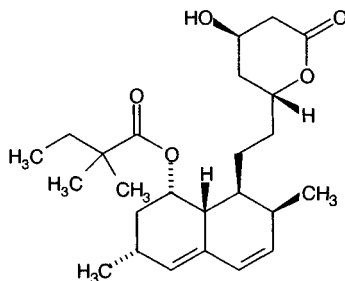
Wiener, H.L.; Kramer, H.K.; Reith, M.E.A. Separation and determination of sertraline and its metabolite, desmethylsertraline, in mouse cerebral cortex by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1990**, 527, 467–472

Simvastatin

Molecular formula: C₂₅H₃₈O₅

Molecular weight: 418.6

CAS Registry No.: 79902-63-9



SAMPLE

Matrix: bile, microsomal incubations, tissue

Sample preparation: Microsomal incubations. 1 mL Microsomal incubation + 1 mL acetone, extract with 2 mL ethyl acetate. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L n-propanol, inject a 20 μ L aliquot. Bile. Adjust pH of bile to 4. Extract 2 mL acidified bile with 10 mL MTBE:ethyl acetate 75:25. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L n-propanol, inject a 20 μ L aliquot. Tissue. Homogenize liver with 4 volumes of water. Extract a 500 μ L aliquot with 500 μ L MeCN. Evaporate the supernatant to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L n-propanol, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m HIBAR Lichrospher 100 CH-18

Mobile phase: Gradient. MeCN:5 mM formic acid from 30:70 to 90:10 over 30 min

Flow rate: 2

Injection volume: 20

Detector: UV 238

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

liver; mouse; human; dog; rat

REFERENCE

Vickers, S.; Duncan, C.A.; Vyas, K.P.; Kari, P.H.; Arison, B.; Prakash, S.R.; Ramjit, H.G.; Pitzenger, S.M.; Stokker, G.; Duggan, D.E. *In vitro* and *in vivo* biotransformation of simvastatin, an inhibitor of HMG CoA reductase. *Drug Metab.Dispos.*, **1990**, *18*, 476-483

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L MeCN:water 60:40, shake at 150 cycles/min for 2 min, centrifuge at 1500 g for 3 min, remove the supernatant and extract the residue again with 400 μ L MeCN, combine the supernatants, centrifuge, evaporate to dryness with a nitrogen stream under vacuum, reconstitute with 200 μ L MeCN:water 25:75, filter (0.45 μ m), inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 20 mm long 40 μ m pellicguard (Supelco)

Column: 250 × 4.6 5 µm ODS Hypersil
Mobile phase: MeCN:25 mM pH 4.5 NaH₂PO₄ 65:35
Flow rate: 1.5
Injection volume: 20
Detector: UV 238

CHROMATOGRAM

Retention time: 7.2 (3.6 hydroxy acid form)
Limit of detection: 15 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS

plasma

REFERENCE

Carlucci, G.; Mazzeo, P.; Biordi, L.; Bologna, M. Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high-performance liquid chromatography with UV detection. *J.Pharm.Biomed.Anal.*, **1992**, *10*, 693–697

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 µL aliquot of a solution in MeOH:100 mM pH 5 KH₂PO₄ 75:25.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18
Column: 50 × 4.6 3 µm Supelcosil LC-18
Mobile phase: MeCN:50 mM pH 3.5 ammonium phosphate 26:74
Column temperature: 50
Flow rate: 1.6
Injection volume: 50
Detector: UV 238

CHROMATOGRAM

Retention time: 4.8 (simvastatin β-hydroxyacid)
Internal standard: simvastatin β-hydroxyacid

OTHER SUBSTANCES

Simultaneous: pravastatin

KEY WORDS

simvastatin β-hydroxyacid is IS

REFERENCE

Iacona, I.; Regazzi, M.B.; Buggia, I.; Villani, P.; Fiorito, V.; Molinaro, M.; Guarnone, E. High-performance liquid chromatography determination of pravastatin in plasma. *Ther.Drug Monit.*, **1994**, *16*, 191–195

SAMPLE

Matrix: solutions
Sample preparation: Centrifuge at 2000 rpm, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Hypersil ODS
Mobile phase: MeCN:water:triethylamine:glacial acetic acid 580:420:1:1

Column temperature: 30

Flow rate: 2

Detector: UV 238

CHROMATOGRAM

Retention time: 10.7 (3.5 hydroxy acid form)

Limit of detection: 10 ng/mL

Limit of quantitation: 25 ng/mL

REFERENCE

Serajuddin, A.T.; Ranadive, S.A.; Mahoney, E.M. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin. *J.Pharm.Sci.*, **1991**, *80*, 830–834

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Vickers, S.; Duncan, C.A.; Chen, I.W.; Rosegay, A.; Duggan, D.E. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. *Drug Metab.Dispos.*, **1990**, *18*, 138–145 [plasma; tissue; mouse; dog; human; SPE; gradient; liver; prostate; testes; adrenal; kidney; lung; spleen; forestom; glandstom]

Somatropin

Molecular formula: C₉₉₀H₁₅₂₈N₂₆₂O₃₀₀S₇

Molecular weight: 21500.0

CAS Registry No.: 9002-72-6, 12629-01-5 (human)

SAMPLE

Matrix: formulations

Sample preparation: Prepare a 1 mg/mL solution in 12.5 mM ammonium bicarbonate, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Guard column: 40 × 6 TSK gel SWXL (Tosoh)

Column: 300 × 7.8 TSK gel G2000SWXL

Mobile phase: 50 mM pH 6.8 Phosphate buffer containing 300 mM NaCl and 0.05% sodium azide (Caution! Sodium azide is carcinogenic, highly toxic, and can form explosive compounds if discharged to the sewer!)

Flow rate: 0.8

Injection volume: 20

Detector: UV 280; RI

CHROMATOGRAM

Retention time: 12.5

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

recombinant; GPC

REFERENCE

Kawashige, M.; Sendo, T.; Otsubo, K.; Aoyama, T.; Oishi, R. Quality evaluation of commercial lyophilized human growth hormone preparations. *Biol.Pharm.Bull.*, **1995**, *18*, 1793–1796

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Zorbax GF-250

Mobile phase: 50 mM ammonium bicarbonate

Flow rate: 0.6

Detector: UV 215

CHROMATOGRAM

Retention time: 13.5, 14.5

KEY WORDS

SEC

REFERENCE

Katakam, M.; Bell, L.N.; Banga, A.K. Effect of surfactants on the physical stability of recombinant human growth hormone. *J.Pharm.Sci.*, **1995**, *84*, 713–716

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 4.6 10 μm RP 300 Aquapore MPLC (Brownlee)

Column: 100 × 4.6 10 μm RP 300 Aquapore MPLC (Brownlee)

Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 54:46 to 43:57 over 22 min.

Detector: UV 214

KEY WORDS

recombinant; cow

REFERENCE

Stevenson, C.L.; Hageman, M.J. Estimation of recombinant bovine somatropin solubility by excluded-volume interaction with polyethylene glycols. *Pharm.Res.*, **1995**, *12*, 1671–1676

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac C4

Mobile phase: Gradient. MeCN:0.05% trifluoroacetic acid from 43:57 to 55:45 over 30 min.

Column temperature: 45

Flow rate: 0.5

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 28

OTHER SUBSTANCES

Simultaneous: somatrem

REFERENCE

Arcelloni, C.; Fermo, I.; Banfi, G.; Pontiroli, A.E.; Paroni, R. Capillary electrophoresis for protein analysis: separation of human growth hormone and human insulin molecular forms. *Anal.Biochem.*, **1993**, *212*, 160–167

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 600 × 7.5 10 μm TSK G3000 SW (Pharmacia)

Mobile phase: 10 mM pH 7.2 K₂HPO₄ containing 300 mM NaCl

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 17 (44K), 21 (22K)

REFERENCE

Arcelloni, C.; Fermo, I.; Banfi, G.; Pontiroli, A.E.; Paroni, R. Capillary electrophoresis for protein analysis: separation of human growth hormone and human insulin molecular forms. *Anal.Biochem.*, **1993**, *212*, 160–167

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 5 μm C4 (Vydac)**Mobile phase:** Isopropanol:500 mM pH 6.5 KH₂PO₄ 29:71**Column temperature:** 45**Flow rate:** 1**Detector:** F ex 295 em 348

CHROMATOGRAM**Retention time:** 25

OTHER SUBSTANCES**Simultaneous:** somatrem

REFERENCE

Oroszlan, P.; Wicar, S.; Teshima, G.; Wu, S.L.; Hancock, W.S.; Karger, B.L. Conformational effects in the reversed-phase chromatographic behavior of recombinant human growth hormone (rhGH) and N-methionyl recombinant human growth hormone (Met-hGH). *Anal.Chem.*, **1992**, *64*, 1623–1631

SAMPLE**Matrix:** solutions**Sample preparation:** Dissolve in 100 mM NaH₂PO₄ 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 NaH₂PO₄ 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:** 3

OTHER SUBSTANCES**Simultaneous:** adrenocorticotropin hormone and fragments, endorphins, lipotropic hormone and fragments, melanotropin, menotropins, prolactin

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, adrenocorticotropin hormone, and β-endorphin. *J.Neurochem.*, **1985**, *44*, 1697–1703

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 1 mg/mL solution in 1% ammonium bicarbonate, inject a 100 μL aliquot.

HPLC VARIABLES**Column:** 250 × 4.1 Synchropak RP-P (Synchrom)**Mobile phase:** Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in n-propanol. A:B from 100:0 to 50:50 over 50 min.

Flow rate: 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 41

REFERENCE

Kohr, W.J.; Keck, R.; Harkins, R.N. Characterization of intact and trypsin-digested biosynthetic human growth hormone by high-pressure liquid chromatography. *Anal.Biochem.*, **1982**, *122*, 348–359

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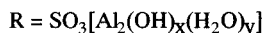
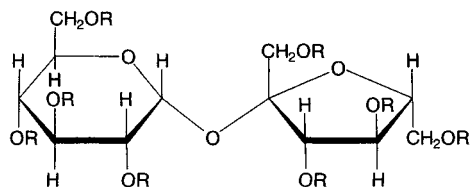
Chang, J.P.; Tucker, R.C.; Ghrist, B.F.; Coleman, M.R. Non-denaturing assay for the determination of the potency of recombinant bovine somatotropin by high-performance size-exclusion chromatography. *J.Chromatogr.A*, **1994**, *675*, 113–122

Sucralfate

Molecular formula: $C_{12}H_{54}Al_6O_{75}S_8$

Molecular weight: 2086.7

CAS Registry No.: 54182-58-0



SAMPLE

Matrix: blood, feces, urine

Sample preparation: Urine. Centrifuge, inject an aliquot as described below. Feces. Homogenize with five times the volume of 0.01 M $(NH_4)_2SO_4$, re-extract the precipitate with 0.01 M $(NH_4)_2SO_4$, combine the supernatants, inject an aliquot as described below. Plasma. Dilute with two volumes water, apply to 20×4 25-40 μm LiChroprep-NH2 equilibrated with 0.01 M $(NH_4)_2SO_4$, wash with 10 mL 0.1 M $(NH_4)_2SO_4$, elute with 1 mL 1 M $(NH_4)_2SO_4$, dilute the eluate with 10 volumes of water, inject an aliquot as described below. Apply up to 5 mL sample to column A with mobile phase A and elute for 10 min then backflush contents of column A onto column B with mobile phase B. Elute column B with mobile phase B and monitor the output. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 40×4 25-40 μm LiChroprep NH2; B 250×4 10 μm LiChrosorb-NH2

Mobile phase: A 0.01 M $(NH_4)_2SO_4$; B 0.8 M $(NH_4)_2SO_4$

Flow rate: 1

Injection volume: 5000

Detector: Radioactivity

CHROMATOGRAM

Retention time: 8-9 (after column switch)

KEY WORDS

column-switching; radiolabeled compound; plasma

REFERENCE

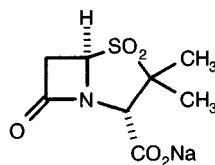
Steiner, K.; Bühring, K.U.; Faro, H.-P.; Garbe, A.; Nowak, H. Sucralfate: pharmacokinetics, metabolism and selective binding to experimental gastric and duodenal ulcers in animals. *Arzneimittelforschung*, 1982, 32, 512-518

Sulbactam

Molecular formula: C₈H₁₁NO₅S

Molecular weight: 233.2

CAS Registry No.: 68373-14-8 (sulbactam), 83031-43-0 (sulbactam benzathine), 69388-79-0 (sulbactam pivoxil), 69388-84-7 (sulbactam sodium)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 400 μ L 10 mg/mL zinc sulfate containing 350 μ g/mL benzoic acid, vortex for 30 s, centrifuge at 5500 g for 5 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.3 5 μ m Nova Pak

Mobile phase: MeOH:pH 6.30 phosphate buffer 5:95

Column temperature: 45

Flow rate: 2

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 1.35

Internal standard: benzoic acid (1.9)

Limit of detection: 10 μ g/mL

OTHER SUBSTANCES

Extracted: tazobactam

KEY WORDS

serum

REFERENCE

Guillaume, Y.; Peyrin, E.; Guinchard, C. Rapid determination of sulbactam and tazobactam in human serum by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *665*, 363–371

SAMPLE

Matrix: blood

Sample preparation: Filter (Amicon MPS-1 with YMT membrane) while centrifuging at 1500 g for 10 min, inject a 20 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μ m Develosil ODS-10

Column: 150 \times 4.6 5 μ m Develosil ODS-5 (Nomura Chemicals)

Mobile phase: MeOH:buffer 1:1.5 (Prepare buffer by dissolving 1.791 g Na₂HPO₄·12H₂O and 0.780 g NaH₂PO₄·2H₂O in 10 L water, add tetrabutylammonium bromide to a final concentration of 5 mM.)

Flow rate: 0.8

Injection volume: 20

Detector: UV 278 following post-column reaction. The column effluent mixed with MeOH: 750 mM NaOH 1:1.5 pumped at 0.2 mL/min and this mixture flowed through a 1 m \times 0.5 mm ID coil to the detector.

CHROMATOGRAM

Retention time: 6

Limit of detection: 25 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Noninterfering: ampicillin, cefoperazone, ticarcillin

KEY WORDS

ultrafiltrate; plasma; post-column reaction

REFERENCE

Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. Improved high-performance liquid chromatographic assay of clavulanic acid and sulbactam by postcolumn alkaline degradation. *J.Liq.Chromatogr.*, **1985**, *8*, 2521–2534

SAMPLE

Matrix: blood, CSF

Sample preparation: 1 mL Plasma or CSF + 200 μ L hydrochloric acid R.P. + 100 μ L 10 μ g/mL pyrogallol in mobile phase + 6 mL diethyl ether, vortex for 1 min, centrifuge at 900 g at 4° for 10 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4.6 10 μ m μ Bondapak C18

Mobile phase: MeOH:water 20:80 with 0.5% glacial acetic acid

Flow rate: 0.75

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 8

Internal standard: pyrogallol (6)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin, ampicillin, cefoxitin

KEY WORDS

plasma

REFERENCE

Fredj, G.; Paillet, M.; Aussel, F.; Brouard, A.; Barreteau, H.; Divine, C.; Micoud, M. Determination of sulbactam in biological fluids by high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *383*, 218–222

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: 1 mL Plasma or saliva or 20 μ L urine + 20 μ L 50 μ g/mL salicylhydroxamic acid + 200 μ g N-hydrochloric acid (?) + 4 mL redistilled diethyl ether, vortex for 30 s, centrifuge at 500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 150 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4.6 Magnosphere C18 (Magnus Scientific, Sandbach, England)

Mobile phase: MeOH:50 mM ammonium phosphate:phosphoric acid 5:95:0.1 (plasma) or 0:99.9:0.1 (urine)

Flow rate: 1

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 7.5 (plasma), 16.5 (urine)

Internal standard: salicylhydroxamic acid (14.0 (plasma), 21.5 (urine))

Limit of quantitation: 200 ng/mL (plasma), 20000 ng/mL (urine)

OTHER SUBSTANCES

Noninterfering: amoxicillin, carbenicillin, cefazolin, cefuroxime, cephalixin, cephaloridine, cephadrine, cloxacillin, flucloxacillin, mecillinam, penicillin G, tetracycline

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Rogers, H.J.; Bradbrook, I.D.; Morrison, P.J.; Spector, R.G.; Cox, D.A.; Lees, L.J. Pharmacokinetics and bioavailability of sultamicillin estimated by high performance liquid chromatography. *J. Anti-microb. Chemother.*, **1983**, *11*, 435-445

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. Filter using Molcut II (Millipore), inject a 50 μ L aliquot of the ultrafiltrate. Urine. Dilute ten-fold with water, filter (Gelman acrylate copolymer 0.45 μ m), inject a 20 μ L aliquot of the filtrate.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m LiChrospher RP-18(e)

Column: 250 \times 4 5 μ m LiChrospher RP-18(e)

Mobile phase: MeOH:20 mM tetrabutylammonium bromide + 5 mM Na₂HPO₄ + 5 mM NaH₂PO₄ 1:1.75

Flow rate: 0.8

Injection volume: 20-50

Detector: UV 270 following post-column reaction. The column effluent mixed with 2 M NaOH and 0.05% sodium hypochlorite solution pumped at 0.1 mL/min in a 400 \times 0.5 mm hollow fiber membrane reactor at 40°, this mixture flowed through a 1400 \times 0.3 mm knitted open tubular reactor at 50° to the UV detector.

CHROMATOGRAM

Retention time: 8

Limit of detection: 5 ng

OTHER SUBSTANCES

Extracted: ampicillin

KEY WORDS

serum; post-column reaction

REFERENCE

Haginaka, J.; Nishimura, Y. Simultaneous determination of ampicillin and sulbactam by liquid chromatography: post-column reaction with sodium hydroxide and sodium hypochlorite using an active hollow-fiber membrane reactor. *J. Chromatogr.*, **1990**, *532*, 87-94

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 1 mL Serum + 1 mL MeCN, shake at 0° for 15 min, centrifuge at 8000 g for 10 min. Add supernatant to 10 mL dichloromethane, shake at 0° for 15 min, centrifuge at 8000 g for 10 min, discard organic layer. Mix 200 µL aqueous layer with 400 µL reagent, after 40 min at 40° inject 40 µL aliquot. Urine. Dilute 10-fold, mix 200 µL with 400 µL reagent, after 40 min at 40° inject 40 µL aliquot. (Reagent was 3.45 g 1,2,4-triazole dissolved in 15 mL water, adjust pH to 6.0 with 4 M NaOH, make up to 25 mL.)

HPLC VARIABLES

Guard column: 50 × 4.6 5µm Spherisorb C-18

Column: 250 × 4.6 5µm Spherisorb C-18

Mobile phase: MeCN:20 mM pH 7.0 phosphate buffer 0.2:99.8

Flow rate: 0.5

Injection volume: 40

Detector: UV 325

CHROMATOGRAM

Retention time: 7

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: ampicillin (with gradient to 25:75 over 25 min)

KEY WORDS

serum; derivatization

REFERENCE

Shah, A.J.; Adlard, M.W.; Stride, J.D. A sensitive assay for clavulanic acid and sulbactam in biological fluids by high-performance liquid chromatography and precolumn derivatization. *J.Pharm. Biomed.Anal.*, **1990**, *8*, 437-443

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute urine ten-fold with water. 50 µL Plasma or 100 µL diluted urine + 150 µL MeCN, vortex for 30 s, incubate at room temperature for 5 min, centrifuge at 1500 g for 10 min. Remove a 100 µL aliquot of the supernatant and add it to 100 µL triazole solution, heat at 50° for 15 min, cool to room temperature, centrifuge at 1500 g for 5 min, inject a 20 µL aliquot of the supernatant. (Triazole solution was 13.81 g 1,2,4-triazole in 60 mL water, pH adjusted to 10.0 ± 0.05 with saturated NaOH solution, made up to 100 mL with water.)

HPLC VARIABLES

Guard column: 30 × 4.6 10 µm Develosil ODS-10

Column: 150 × 4.6 5 µm Develosil ODS-5

Mobile phase: MeCN:5 mM tetrabutylammonium bromide + 1 mM Na₂HPO₄ + 1 mM NaH₂PO₄ 25:75

Column temperature: 50

Flow rate: 1

Injection volume: 20

Detector: UV 326

CHROMATOGRAM

Retention time: 7

Limit of detection: 200 ng/mL (plasma); 1 µg/mL (urine)

OTHER SUBSTANCES

Noninterfering: degradation products, amoxicillin, ampicillin, cefoperazone, penicillin G

KEY WORDS

plasma; human; rat; derivatization

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: 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 \times 300 $\mu\text{Bondapak C18}$

Mobile phase: MeCN:MeOH:10 mM pH 2.6-2.7 phosphate buffer 7:14:79 containing 5 mM tetrabutylammonium hydrogen sulfate

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 5.79

Internal standard: cimetidine (3.27)

OTHER SUBSTANCES

Simultaneous: ampicillin, aztreonam

KEY WORDS

stability-indicating; saline; injections

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: Dilute injection with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: $\mu\text{Bondapak C18}$

Mobile phase: MeCN:buffer 17.5:82.5 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 5.0 with concentrated phosphoric acid.)

Column temperature: 25

Flow rate: 2

Injection volume: 10

Detector: UV 230

CHROMATOGRAM

Retention time: 10.1

OTHER SUBSTANCES**Simultaneous:** ampicillin

KEY WORDS

injections

REFERENCE

Mushinsky, R.F.; Reynolds, M.L.; Nicholson, C.A.; Crider, L.L.; Forcier, G.A. Stability of sulbactam/ampicillin in diluents for parenteral administration. *Rev.Infect.Dis.*, **1986**, *8 Suppl 5*, S523-S527

SAMPLE**Matrix:** urine**Sample preparation:** Dilute 10-fold with water, filter (0.45 μ m acrylate copolymer), inject a 20 μ L aliquot.

HPLC VARIABLES**Guard column:** 30 \times 4.6 10 μ m Develosil ODS-10**Column:** 150 \times 4.6 5 μ m Develosil ODS-5 (Nomura Chemicals)**Mobile phase:** MeOH:buffer 1:2 (Prepare buffer by dissolving 1.791 g Na₂HPO₄.12H₂O and 0.780 g NaH₂PO₄.2H₂O in 10 L water, add tetrabutylammonium bromide to a final concentration of 5 mM.)**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 272 following post-column reaction. The column effluent mixed with MeOH: 750 mM NaOH 1:2 pumped at 0.2 mL/min and this mixture flowed through a 1 m \times 0.5 mm ID coil to the detector.

CHROMATOGRAM**Retention time:** 9**Limit of detection:** 500 ng/mL**Limit of quantitation:** 1 μ g/mL

OTHER SUBSTANCES**Noninterfering:** ampicillin, cefoperazone, ticarcillin

KEY WORDS

post-column reaction

REFERENCE

Haginaka, J.; Wakai, J.; Yasuda, H.; Uno, T.; Nakagawa, T. Improved high-performance liquid chromatographic assay of clavulanic acid and sulbactam by postcolumn alkaline degradation. *J.Liq.Chromatogr.*, **1985**, *8*, 2521-2534

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Bawdon, R.E.; Madsen, P.O. High-pressure liquid chromatographic assay of sulbactam in plasma, urine, and tissue. *Antimicrob.Agents Chemother.*, **1986**, *30*, 231-233

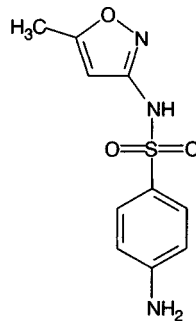
Haginaka, J.; Yasuda, H.; Uno, T.; Nakagawa, T. Sulbactam: alkaline degradation and determination by high-performance liquid chromatography. *Chem.Pharm.Bull.*, **1984**, *32*, 2752-2758

Sulfamethoxazole

Molecular formula: C₁₀H₁₁N₃O₃S

Molecular weight: 253.3

CAS Registry No.: 723-46-6



SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bakerbond C18 SPE cartridge with MeOH and 50 mM pH 5.5 citrate buffer. 500 μ L Serum + 17 μ L 600 μ g/mL sulfamethazine in MeCN: water 20:80 + 500 μ L 50 mM pH 5.5 citrate buffer, vortex, add to the SPE cartridge, wash twice with 50 mM pH 5.5 citrate buffer, air dry, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μ L mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 Guard-Pak

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:1% acetic acid 18:82

Flow rate: 2.5

Injection volume: 100

Detector: UV 240

CHROMATOGRAM

Retention time: 8.93

Internal standard: sulfamethazine (4.19)

OTHER SUBSTANCES

Extracted: trimethoprim

KEY WORDS

serum; SPE

REFERENCE

Moore, K.H.P.; Brouwer, K.L.R. High-performance liquid chromatographic evaluation of the effect of heat treatment on trimethoprim and sulfamethoxazole stability in serum. *Ther. Drug Monit.*, **1995**, *17*, 356-360

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma, whole blood, or red blood cells + 100 μ L MeOH + 500 μ L water + 100 μ L buffer + 6 mL ethylene dichloride, shake on an orbital mixer for 20 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness at 60° on a vortex evaporator, reconstitute the residue in 200 μ L mobile phase, inject a 20 μ L aliquot. (Buffer was prepared by adding 100 μ L acetic acid to 9.9 mL phosphate buffer, pH 3.40.)

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak 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: 8

Internal standard: sulfamethoxazole

OTHER SUBSTANCES

Extracted: sulfadoxine

Simultaneous: amodiaquine, chloroquine, dapsone, primaquine, pyrimethamine, quinidine, quinine, sulfalene

KEY WORDS

plasma; whole blood; red blood cells; sulfamethoxazole is IS

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: blood

Sample preparation: Condition a 3 mL C18 Bond Elut SPE cartridge with 500 μ L MeOH then 1 mL wash solution. 500 μ L Serum + 500 μ L wash solution, vortex for 3 s, add 25 μ L 240 μ g/mL p-nitrophenol in MeOH, vortex for 3 s, add to the SPE cartridge, wash with 1 mL wash solution, dry with vacuum applied for 30 s, elute with two 500 μ L aliquots of MeOH:triethylamine 10:1. Combine the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute in 200 μ L mobile phase, inject a 50-100 μ L aliquot. (Wash solution was 7 mL 3 M HCl + 1.6 g 1-octanesulfonic acid + 150 mL 100 mM disodium citrate made up to 800 mL with water, pH adjusted to 3.00 with 3 M HCl.)

HPLC VARIABLES

Guard column: 20 \times 2 40 μ m Upchurch pellicular C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:buffer 24:76 with 0.8 g 1-octanesulfonic acid (Buffer was 7 mL 3 M HCl plus 150 mL 100 mM disodium citrate made up to 760 mL with water, pH adjusted to 3.00 with 3 M HCl.)

Flow rate: 1.5

Injection volume: 50-100

Detector: UV 230

CHROMATOGRAM

Retention time: 6.8

Internal standard: p-nitrophenol (9.3)

Limit of quantitation: 250 ng

OTHER SUBSTANCES

Extracted: N⁴-acetylsulfamethoxazole, trimethoprim

KEY WORDS

serum; SPE

REFERENCE

Laizure, S.C.; Holden, C.L.; Stevens, R.C. Ion-paired high-performance liquid chromatographic separation of trimethoprim, sulfamethoxazole and N⁴-acetylsulfamethoxazole with solid-phase extraction. *J.Chromatogr.*, **1990**, *528*, 235–242

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 200 μ L MeOH + 100 μ L 50 μ g/mL sulfapyridine in MeOH + 1 mL 1 M pH 4.7 sodium acetate buffer + 8 mL dichloromethane, shake vigorously for 2 min, centrifuge at 1000 g at 10° for 10 min. Remove the organic layer and evaporate it to dryness at 50° under nitrogen, take up the residue in 250 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 75 \times 4.6 Whatman CO:Pell

Column: 250 \times 3.2 10 μ m Spherisorb ODS

Mobile phase: MeOH:50 mM pH 7.4 phosphate buffer 20:80

Flow rate: 1.2

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 2.7

Internal standard: sulfapyridine (5.2)

OTHER SUBSTANCES

Extracted: acetylsulfamethoxazole

KEY WORDS

plasma

REFERENCE

Astbury, C.; Dixon, J.S. Rapid method for the determination of either plasma sulfapyridine or sulfamethoxazole and their acetyl metabolites using high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *414*, 223–227

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μ L 5 μ g/mL sulfafurazole in MeOH:water 50:50 + 200 μ L 1 M pH 6.8 sodium phosphate buffer + 6 mL ethyl acetate, shake for 15 min, centrifuge at 600 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 175 μ L MeOH:water 20:80, vortex for 1 min, add 25 μ L 40% trichloroacetic acid in 100 mM hydrochloric acid, vortex for 30 s, centrifuge at 8300 g for 5 min, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.5 μ m LiChrosorb RP18

Mobile phase: MeCN:150 mM pH 4.85 ammonium phosphate buffer 12.6:87.4

Flow rate: 1.5

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 23

Internal standard: sulfafurazole (21)

Limit of detection: 80 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, N-acetylsulfamethoxazole, trimethoprim

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Van der Steuijt, K.; Sonneveld, P. Concurrent analysis of methotrexate, trimethoprim, sulfamethoxazole and their major metabolites in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *422*, 328-333

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 50 μ L 50 μ g/mL 4-dimethylaminobenzaldehyde in MeOH, vortex for 10 s, add 250 μ L 60% perchloric acid, vortex for 10 s, centrifuge at 3000 rpm for 10 min, inject a 10-25 μ L aliquot of the clear supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Hypersil ODS

Mobile phase: MeCN:buffer 25:75 (Buffer contained 50 mM Na₂HPO₄ adjusted to pH 2 with orthophosphoric acid.)

Flow rate: 2

Injection volume: 10-25

Detector: UV 254

CHROMATOGRAM

Retention time: 3

Internal standard: 4-dimethylaminobenzaldehyde (7)

Limit of quantitation: 100 ng/mL

KEY WORDS

serum

REFERENCE

Hung, C.T.; Perrier, D.G. Determination of trimethoprim and sulfamethoxazole in serum by reversed-phase and ion pair HPLC. *J.Liq.Chromatogr.*, **1985**, *8*, 521-536

SAMPLE

Matrix: blood, CSF, gastric fluid, urine

Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH₂PO₄. 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:** 9.5**Internal standard:** heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, 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, 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, peritoneal fluid, synovial fluid, tissue, urine

Sample preparation: Condition a C18 SPE cartridge (J.T. Baker) with 1 mL MeOH, 1 mL water, and 1 mL 100 mM pH 4.5 acetate buffer. CSF, peritoneal fluid, serum, synovial fluid, tissue. Homogenize (TenBroeck tissue grinder) endometrial tissue with saline, centrifuge at 510 g, remove supernatant. Centrifuge fluids at 510 g. 1 mL Sample + 1 mL 100 mM acetate buffer, mix, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeCN:MeOH:100 mM acetate buffer 12.5:12.5:75, add 12.5 ng ormetoprim, inject an aliquot. Urine. 1 mL Urine + 1 mL MeCN, centrifuge at 510 g for 10 min. Remove 25 μ L of the supernatant and add it to 1 mL 100 mM acetate buffer, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeCN:MeOH:100 mM acetate buffer 12.5:12.5:75, add 12.5 ng ormetoprim, inject an aliquot.

HPLC VARIABLES**Column:** C18 (Rainin)**Mobile phase:** MeCN:MeOH:50 mM phosphate buffer 12.5:12.5:75, pH 3.0**Flow rate:** 1.5**Detector:** UV 280

CHROMATOGRAM**Retention time:** 11**Internal standard:** ormetoprim (8)**Limit of detection:** 50 ng/mL

OTHER SUBSTANCES**Extracted:** trimethoprim

KEY WORDS

serum; endometrium; SPE; horse; pharmacokinetics

REFERENCE

Brown, M.P.; Gronwall, R.; Castro, L. Pharmacokinetics and body fluid and endometrial concentrations of trimethoprim-sulfamethoxazole in mares. *Am.J.Vet.Res.*, **1988**, 49, 918–922

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: 50 μ L Serum, urine, or CSF + 50 μ L 100 μ g/mL antipyrine in MeOH, vortex for 15 s, centrifuge at 10000 g for 2 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 37-50 μ m μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:buffer 35:65 (Buffer was 97% 67 mM KH_2PO_4 + 3% 67 mM Na_2HPO_4 and the pH was adjusted to 3.5 with phosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 8.0

Internal standard: antipyrine (11.0)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: N-acetylsulfamethoxazole, trimethoprim

KEY WORDS

serum

REFERENCE

Weber, A.; Opheim, K.E.; Siber, G.R.; Ericson, J.F.; Smith, A.L. High-performance liquid chromatography quantitation of trimethoprim, sulfamethoxazole, and N4-acetylsulfamethoxazole in body fluids. *J.Chromatogr.*, **1983**, *278*, 337-345

SAMPLE

Matrix: blood, milk

Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, 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 50 μ L water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μ L aliquot of the clear layer and add it to 100 μ L 1 mg/mL fluorecamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m Nova-Pak C18

Mobile phase: MeCN: 10 mM KH_2PO_4 30:70

Flow rate: 1

Injection volume: 50

Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 12.5

Internal standard: p-aminobenzoic acid (5.5)

Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamonomethoxine, sulfathiazole

KEY WORDS

cow; serum; derivatization

REFERENCE

Tsai, C.-E.; Kondo, F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk. *J.AOAC Int.*, **1995**, *78*, 674–678

SAMPLE

Matrix: blood, tissue

Sample preparation: 1 mL Serum or homogenized tissue + 4 mL MeCN, vortex, 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 50 μ L water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL sulfadiazine in 0.01% trichloroacetic acid, shake, add 100 μ L hexane, shake, centrifuge at 1000 g for 15 min. Remove a 500 μ L aliquot of the clear aqueous layer and add it to 100 μ L 1 mg/mL fluorescamine in MeCN (freshly prepared), shake by hand for 1 min, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m Nova-Pack C18

Mobile phase: MeCN:10 mM KH_2PO_4 30:70

Flow rate: 1

Injection volume: 50

Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 14.1

Internal standard: sulfadiazine (7.1)

Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: sulfadimethoxine, sulfamethazine, sulfamonomethoxine

KEY WORDS

serum; pig; derivatization; kidney; muscle; liver

REFERENCE

Tsai, C.-E.; Kondo, F. A sensitive high-performance liquid chromatographic method for detecting sulfonamide residues in swine serum and tissues after fluorescamine derivatization. *J.Liq.Chromatogr.*, **1995**, *18*, 965–976

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μ L Plasma + 100 μ L MeCN, centrifuge at 3000 g for 5 min, inject 20 μ L of the supernatant. Urine. 7 mL Urine + 50 μ L diethylamine, centrifuge at 3000 g for 5 min, dilute the supernatant 1:9 with 200 mM pH 5.0 K_2HPO_4 buffer, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 75 \times 3.1 Chrompack pellicular reversed phase (cat. no. 28653)

Column: 250 \times 4.6 5 μ m Spherisorb ODS

Mobile phase: Gradient. MeCN:DMF:buffer A:B:C. Over 30 min from A:B:C 1:3:96 to 20:10:70, then over 3 min to 1:3:96, keep at 1:3:96 for 2 min before next injection. (Buffer was 0.9 g 85% phosphoric acid and 0.225 g tetramethylammonium chloride in 1 L water, pH 2.1.)

Flow rate: 1.2

Injection volume: 20

Detector: UV 271

CHROMATOGRAM

Retention time: 23

Limit of detection: 35 ng/mL (plasma)

Limit of quantitation: 100 ng/mL (plasma); 1500 ng/mL (urine)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Vree, T.B.; van der Ven, A.J.A.M.; Verwey-van Wissen, C.P.W.G.M.; van Ewijk-Beneken Kolmer, E.W.J.; Swolfs, A.E.M.; van Galen, P.M.; Amadajais-Groenen, H. Isolation, identification and determination of sulfamethoxazole and its known metabolites in human plasma and urine by high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, *658*, 327–340

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: μ Bondapak C18

Mobile phase: MeCN:water 20:80

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Also analyzed: sulfadiazine, sulfamerazine, sulfanilamide

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 sulfonamides and trimethoprim. *J.Antimicrob.Chemother.*, **1986**, *17*, 489–498

SAMPLE

Matrix: eggs, food, milk

Sample preparation: Honey. Dissolve 1 g honey in 10 mL water, homogenize, filter (0.45 μ m), inject a 50 μ L aliquot. Milk, eggs. 5 mL Milk or 0.4 g lyophilized eggs + 10 mL trichloroacetic acid solution (so as to give a final trichloroacetic acid concentration of 3%), homogenize, centrifuge at 5000 rpm for 5 min. Re-extract the residue with 10 mL 3% trichloroacetic acid. Combine the aqueous phases and make up to 25 mL with trichloroacetic acid solution, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (Wash column with MeCN:ethyl acetate 5:95 at the end of each day.)

Flow rate: 1

Injection volume: 50

Detector: UV 260

CHROMATOGRAM

Retention time: 16

Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfaguanidine, sulfapyridine, sulfathiazole

KEY WORDS

honey

REFERENCE

Viñas, P.; López Erroz, C.; Hernández Canals, A.; Hernández Córdoba, M. Liquid chromatographic analysis of sulfonamides in foods. *Chromatographia*, **1995**, *40*, 382–386

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Homogenize 3 g milk and 500 μL 30% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot. Fish, eggs. Homogenize (Ultra-Turrax) 3 g fish or 4 g eggs with 4 mL 3% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Spherisorb ODS-2

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (At the end of each day wash with MeCN:ethyl acetate 5:95.)

Flow rate: 0.5

Injection volume: 50

Detector: F ex 302 em 412 following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and with reagent 2 pumped at 0.25 mL/min and this mixture flowed through a 2.5 m × 0.8 mm i.d. PTFE coil at 40° to the detector. (Reagent 1 was 10 mM o-phthalaldehyde in EtOH:700 mM phosphoric acid 2:98. Reagent 2 was 20 mM β-mercaptoethanol in EtOH:700 mM phosphoric acid 2:98.)

CHROMATOGRAM

Retention time: 26

Limit of detection: 11 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfaguanidine, sulfanilamide, sulfapyridine

Noninterfering: sulfathiazole

KEY WORDS

post-column reaction

REFERENCE

Viñas, P.; Erroz, C.L.; Campillo, N.; Hernández-Córdoba, M. Determination of sulphonamides in foods by liquid chromatography with postcolumn fluorescence derivatization. *J.Chromatogr.A*, **1996**, *726*, 125–131

SAMPLE**Matrix:** formulations

Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μ L 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES**Guard column:** 35 \times 4.6 C18 (Scharlau)**Column:** 125 \times 4.6 5 μ m Spherisorb ODS-2 C18**Mobile phase:** Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer**Flow rate:** 1**Injection volume:** 20**Detector:** UV 490

CHROMATOGRAM**Retention time:** 7.5**Limit of detection:** 100 ng/mL

OTHER SUBSTANCES

Simultaneous: sulfacetamide, sulfadiazine, sulfaguandidine, sulfamerazine, sulfamethizole, sulfanilamide, sulfathiazole

Noninterfering: benzocaine

KEY WORDS

tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE

Garcia-Alvarez-Coque, M.C.; Simo-Alfonso, E.F.; Ramis-Ramos, G.; Esteve-Romero, J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization. *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245

SAMPLE**Matrix:** microsomal incubations

Sample preparation: Add a volume of 15% perchloric acid equal to 10% of the volume of the microsomal incubation, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES**Guard column:** 30 \times 4.6 5 μ m Ultracarb ODS(30) C18 (Phenomenex)**Column:** 150 \times 4.6 5 μ m Ultracarb ODS(30) C18 (Phenomenex)**Mobile phase:** MeCN:water:glacial acetic acid:triethylamine 20:80:1:0.05**Flow rate:** 1.5**Detector:** UV 254

CHROMATOGRAM**Retention time:** 8.7

OTHER SUBSTANCES**Extracted:** metabolites

REFERENCE

Cribb, A.E.; Spielberg, S.P.; Griffin, G.P. N⁴-Hydroxylation of sulfamethoxazole by cytochrome P450 of the cytochrome P4502C subfamily and reduction of sulfamethoxazole hydroxylamine in human and rat hepatic microsomes. *Drug Metab.Dispos.*, **1995**, *23*, 406-414

SAMPLE**Matrix:** milk

Sample preparation: 500 μ L Milk + 2 g C18 material + 10 μ L MeOH + 10 μ L 12.5 μ g/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μ L pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μ L MeOH and 400 μ L 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μ m), inject a 20 μ L aliquot. (C18 material was Analytichem 40 μ m 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES**Column:** 75 \times 4 3 μ m Supelcosil LC-18**Mobile phase:** MeCN:17 mM orthophosphoric acid 10:90**Column temperature:** 45**Flow rate:** 1 for 5 min then 2 for remainder of run**Injection volume:** 20**Detector:** UV 270

CHROMATOGRAM**Retention time:** 7**Internal standard:** sulfamerazine (3)**Limit of detection:** 62.5 ng/mL

OTHER SUBSTANCES**Extracted:** sulfadiazine, sulfadimethoxine, sulfamethazine, sulfanilamide, sulfathiazole, sulfisoxazole

KEY WORDS

SPE; matrix solid-phase dispersion

REFERENCE

Long, A.R.; Short, C.R.; Barker, S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk. *J.Chromatogr.*, **1990**, *502*, 87-94

SAMPLE**Matrix:** reaction mixtures

Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μ L 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 35:65**Flow rate:** 1**Injection volume:** 20**Detector:** UV 270

CHROMATOGRAM**Retention time:** 6.6**Limit of detection:** 200 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 × 0.35 5 μm Vydac IDI-TP C18 TMS capped**Mobile phase:** Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)**Column temperature:** 30**Flow rate:** 0.006**Injection volume:** 1**Detector:** UV 270

CHROMATOGRAM**Retention time:** 42

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxy-pyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinolaxine, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci, M.C.; Cross, R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming. *J.Liq.Chromatogr.& Rel.Technol.*, **1996**, 19, 547–564

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped

Mobile phase: Gradient. MeOH:1 mM pH 2.72 phosphate buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min.

Column temperature: 30

Flow rate: 0.006

Injection volume: 1

Detector: UV 270

CHROMATOGRAM

Retention time: 46

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinolaxine, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci, M.C.; Cross, R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions. *J.Liq.Chrom. Rel.Technol.*, **1996**, *19*, 365–381

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 µm Ultrasphere C18

Mobile phase: MeCN:water 27:73, adjusted to pH 3.5 with acetic acid

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 2.4

OTHER SUBSTANCES

Simultaneous: metabolites

REFERENCE

Nakamura, H.; Uetrecht, J.; Cribb, A.E.; Miller, M.A.; Zahid, N.; Hill, J.; Josephy, P.D.; Grant, D.M.; Spielberg, S.P. *In vitro* formation, disposition and toxicity of N-acetoxysulfamethoxazole, a potent mediator of sulfamethoxazole toxicity. *J.Pharmacol.Exp.Ther.*, **1995**, *274*, 1099–1104

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:buffer 27:73 (Buffer was 0.1% trifluoroacetic acid adjusted to pH 3 with ammonium hydroxide.)

Flow rate: 1

Injection volume: 20

Detector: UV 267

CHROMATOGRAM

Retention time: k' 2.8

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: Prepare a solution in mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Nucleosil 5C18

Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 44.0

OTHER SUBSTANCES

Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE

Nishikawa, M.; Takahashi, Y.; Ishihara, Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues. *J.Liq.Chromatogr.*, **1993**, 16, 4031–4047

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 2.1 5 μ m 201TP (Vydac)

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 40:60 over 20 min.

Flow rate: 0.2

Injection volume: 5

Detector: UV 270; MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM

Retention time: 14.86

OTHER SUBSTANCES

Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE

Pleasant, S.; Blay, P.; Quilliam, M.A.; O'Hara, G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh. *J.Chromatogr.*, **1991**, *558*, 155–173

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PCX-500 (Dionex)

Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 6.8

OTHER SUBSTANCES

Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfanilic acid, sulfathiazole, sulfisoxazole

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 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 254

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES

Simultaneous: sulfachlorpyridine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfisoxazole

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 solution in MeOH.

HPLC VARIABLES**Column:** 300 × 3.9 μBondapak C18**Mobile phase:** MeCN:water:acetic acid 12.5:86.5:1**Flow rate:** 1.6**Injection volume:** 10**Detector:** UV 254

CHROMATOGRAM**Retention time:** 16

OTHER SUBSTANCES**Simultaneous:** sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCERoos, R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides. *J.Assoc.Off.Anal.Chem.*, **1981**, 64, 851–854

SAMPLE**Matrix:** tissue**Sample preparation:** Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxyppyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH₂PO₄, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Spherisorb C18 ODS**Mobile phase:** MeCN:10 mM pH 4.6 ammonium acetate 28:72:1.2**Flow rate:** 1.2**Injection volume:** 20-50**Detector:** UV 265; MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320°

CHROMATOGRAM**Retention time:** 11.7**Internal standard:** sulfaethoxyppyridazine (12.8)

OTHER SUBSTANCES**Extracted:** sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxyppyridazine, sulfathiazole

KEY WORDS

cow; pig; muscle; liver; SPE

REFERENCEBoison, J.O.; Keng, L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry. *J.AOAC Int.*, **1995**, 78, 651–658

SAMPLE

Matrix: tissue

Sample preparation: Extract with supercritical carbon dioxide into a MeOH solution.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18

Column: 150 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeOH:100 mM KH₂PO₄ adjusted to pH 4.5 with phosphoric acid 28:72

Flow rate: 0.5

Detector: UV 270

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Simultaneous: N⁴-acetylsulfamethoxazole, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxy pyridazine

KEY WORDS

chicken; pig; liver; muscle; SFE

REFERENCE

Cross, R.F.; Ezzell, J.L.; Richter, B.E. The supercritical fluid extraction of polar drugs (sulphonamides) from inert matrices and meat animal products. *J.Chromatogr.Sci.*, **1993**, *31*, 162–169

SAMPLE

Matrix: tissue

Sample preparation: 1-3 g Tissue + 2 (liver) or 3 (muscle) mL 0.7% trichloroacetic acid in acetone, mix in Whirlmixer, sonicate for 10 min at 40°, add 2 mL 10 mM pH 6 Na₂HPO₄, sonicate for 5 min, add 100 μL 500 mM NaOH, add 9 (muscle) or 10 (liver) mL dichloromethane, mix thoroughly for 1 min, centrifuge at 2240 g for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness at 40° under a stream of nitrogen. Dissolve the residue in 400 (muscle) or 800 (liver) μL MeCN:10 mM pH 2.8 phosphate buffer 20:80, sonicate, wash with 1 mL hexane. Sonicate the aqueous phase for 1 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant.

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:buffer 20:80 (tissue) with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)

Flow rate: 0.9

Injection volume: 10

Detector: UV 270

CHROMATOGRAM

Retention time: 18

Internal standard: sulfamethoxazole

OTHER SUBSTANCES

Extracted: sulfadiazine, trimethoprim

KEY WORDS

fish; salmon; trout; sulfamethoxazole is IS; muscle; liver

REFERENCE

Hormazabal, V.; Rogstad, A. Simultaneous determination of sulphadiazine and trimethoprim in plasma and tissues of cultured fish for residual and pharmacokinetic studies. *J.Chromatogr.*, **1992**, *583*, 201-207

SAMPLE

Matrix: tissue

Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μ L Aqueous layer + 250 μ L 3.5 M sodium acetate solution, vortex, add 100 μ L 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Chemcosorb 5-ODS-H

Mobile phase: MeCN:2% acetic acid 5:3

Column temperature: 55

Flow rate: 1

Injection volume: 10

Detector: F ex 405 em 495

CHROMATOGRAM

Retention time: 12

Limit of detection: 5 pg/g

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamonomethoxine, sulfaquinoxaline, sulfisomidine

KEY WORDS

cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE

Takeda, N.; Akiyama, Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products. *J.Chromatogr.*, **1991**, *558*, 175-180

SAMPLE

Matrix: tissue

Sample preparation: 500 mg Tissue + 2 g C18 material + 10 μ L MeOH, let stand for 2 min, grind gently with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μ L pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen at 40°, dissolve the residue in 500 μ L mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter supernatant (0.45 μ m), inject a 25 μ L aliquot. (C18 material was Analytichem 40 μ m 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES

Column: 300 \times 4 MicroPak ODS

Mobile phase: MeCN:17 mM orthophosphoric acid 35:65

Column temperature: 40

Flow rate: 1
Injection volume: 25
Detector: UV 270

CHROMATOGRAM

Retention time: 6
Internal standard: sulfamethoxazole

OTHER SUBSTANCES

Extracted: sulfadimethoxine

KEY WORDS

muscle; fish; SPE; catfish; matrix solid-phase dispersion; sulfamethoxazole is IS

REFERENCE

Long, A.R.; Hsieh, L.C.; Malbrough, M.S.; Short, C.R.; Barker, S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of sulfadimethoxine in catfish (*Ictalurus punctatus*) muscle tissue. *J.Assoc.Off.Anal.Chem.*, **1990**, 73, 868–871

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μ L 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 35 \times 4.6 C18 (Scharlau)

Column: 125 \times 4.6 5 μ m Spherisorb ODS-2 C18

Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer

Flow rate: 1

Injection volume: 20

Detector: UV 490

CHROMATOGRAM

Retention time: 8.5

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: sulfamethizole, sulfathiazole

Interfering: sulfadiazine, sulfaguanidine

KEY WORDS

derivatization

REFERENCE

Simó-Alfonso, E.F.; Ramis-Ramos, G.; García-Alvarez-Coque, M.C.; Esteve-Romero, J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography. *J.Chromatogr.B*, **1995**, 670, 183–187

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Barker, S.A.; Long, A.R. Preparation of milk samples for immunoassay and liquid chromatographic screening using matrix solid-phase dispersion. *JAOAC Int.*, **1994**, 77, 848–854 [MSPD; infant for-

- mula; also albendazole, chloramphenicol, chlorsulon, chlortetracycline, fenbendazole, furazolidone, mebendazole, oxfendazole, oxytetracycline, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfanilamide, sulfathiazole, sulfisoxazole, tetracycline, thiabendazole; LOD 0.1-2 ng]
- Bonazzi, D.; Andrisano, V.; Di Pietra, A.M.; Cavrini, V. Analysis of trimethoprim-sulfonamide drug combinations in dosage forms by UV spectroscopy and liquid chromatography (HPLC). *Farmaco*, **1994**, *49*, 381-386 [simultaneous sulfadiazine, sulfamethoxyypyridazine, trimethoprim]
- Lee, B.L.; Delahunty, T.; Safrin, S. The hydroxylamine of sulfamethoxazole and adverse reactions in patients with acquired immunodeficiency syndrome. *Clin.Pharmacol.Ther.*, **1994**, *56*, 184-189 [urine; extracted N-acetylsulfamethoxazole, sulfamethoxazole hydroxylamine; dinitrobenzylpyridine (IS); normal phase; LOD 5 µg/mL]
- Mokry, M.; Klimes, J.; Zahradnicek, M. HPLC analysis of some sulfonamides in selected pharmaceutical formulations. *Pharmazie*, **1994**, *49*, 333-335 [tablets; injections; acetanilide (IS); simultaneous trimethoprim; also phenacetin, phenazone, phenobarbital, phthalylsulfathiazole, sulfadimidine, sulfamethoxydiazine, sulfamoxole, sulfisoxazole]
- Shah, K.P.; Chang, M.; Riley, C.M. Automated analytical systems for drug development studies. II-A system for dissolution testing. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1519-1527 [tablets; also acetaminophen, trimethoprim]
- Mengelters, M.J.B.; Polman, A.M.M.; Aerts, M.M.L.; Kuiper, H.A.; Van Miert, A.S.J.P.A.M. Determination of sulfadimethoxine, sulfamethoxazole, trimethoprim and their main metabolites in lung and edible tissues from pigs by multi-dimensional liquid chromatography. *J.Liq.Chromatogr.*, **1993**, *16*, 257-278 [muscle; kidney; liver; LOD 10-50 ng/g; column temp 30; column-switching]
- Endoh, Y.S.; Takahashi, Y.; Nishikawa, M. HPLC determination of sulfonamides, their N4-acetyl metabolites and diaminopyrimidine coccidiostats in chicken tissues. *J.Liq.Chromatogr.*, **1992**, *15*, 2091-2110 [skin; plasma; muscle; liver; kidney; LOD 20-50 ng/g; also N-acetyldiaveridine, N-acetylsulfadiazine, N-acetylsulfadimethoxine, N-acetylsulfamethoxazole, N-acetylsulfamonomethoxine, N-acetylsulfaquinoxaline, diaveridine, ormetoprim, sulfadiazine, sulfadimethoxine, sulfamonomethoxine, sulfaquinoxaline, trimethoprim]
- Avgerinos, A.; Athanasiou, G.; Malamataris, S. Rapid simultaneous determination of trimethoprim, sulfamethoxazole and acetylsulfamethoxazole in human plasma and urine by high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1991**, *9*, 507-510
- Nelis, H.J.; Leger, F.; Sorgeloos, P.; De Leenheer, A.P. Liquid chromatographic determination of efficacy of incorporation of trimethoprim and sulfamethoxazole in brine shrimp (*Artemia* spp.) used for prophylactic chemotherapy of fish. *Antimicrob.Agents Chemother.*, **1991**, *35*, 2486-2489
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- Varoquaux, O.; Cordonnier, P.; Advenier, C.; Pays, M. Simultaneous HPLC determination of trimethoprim, sulfamethoxazole and its N4-acetyl metabolite in biological fluids. *Methodol.Surv. Biochem.Anal.*, **1990**, *20*, 123-130
- Cross, R.F. Narrow-bore high-performance liquid chromatography separations of 22 sulfonamides. *J.Chromatogr.*, **1989**, *478*, 422-428 [simultaneous phthalyl sulfathiazole, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguandine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxyypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole]
- Long, A.R.; Hsieh, L.C.; Malbrough, M.S.; Short, C.R.; Barker, S.A. A multiresidue method for the isolation and liquid chromatographic determination of seven sulfonamides in infant formula. *J.Liq.Chromatogr.*, **1989**, *12*, 1601-1612 [extracted sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfathiazole, sulfisoxazole; column temp 45; SPE]
- Mengelters, M.J.B.; Oorsprong, M.B.M.; Kuiper, H.A.; Aerts, M.M.L.; Van Gogh, E.R.; Van Miert, A.S.J.P.A.M. Determination of sulfadimethoxine, sulfamethoxazole, trimethoprim and their main metabolites in porcine plasma by column switching HPLC. *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1765-1776

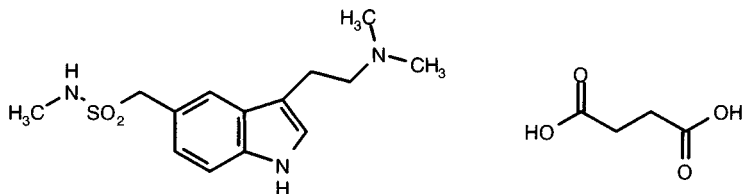
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- Robinson, J.W. Normal phase liquid chromatographic determination of sulfamethoxazole in tablets: collaborative study. *J.Assoc.Off.Anal.Chem.*, **1985**, 68, 88–91
- Spreux-Varoquaux, O.; Chapalain, J.P.; Cordonnier, P.; Advenier, C.; Pays, M.; Lamine, L. Determination of trimethoprim, sulfamethoxazole and its N4-acetyl metabolite in biological fluids by high-performance liquid chromatography. *J.Chromatogr.*, **1983**, 274, 187–199
- Cairnes, D.A.; Evans, W.E. High-performance liquid chromatographic assay of methotrexate, 7-hydroxymethotrexate, 4-deoxy-4-amino-N10-methylptericoic acid and sulfamethoxazole in serum, urine and cerebrospinal fluid. *J.Chromatogr.*, **1982**, 231, 103–110
- Essers, L.; Korte, H. Comparison of the conventional methods and high-performance liquid chromatography for the determination of trimethoprim, sulfamethoxazole and its metabolite in serum. *Chemotherapy (Basel)*, **1982**, 28, 247–252
- Gochin, R.; Kanfer, I.; Haigh, J.M. Simultaneous determination of trimethoprim, sulfamethoxazole and N4-acetylsulfamethoxazole in serum and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1981**, 223, 139–145
- Ascalone, V. Assay of trimethoprim, sulfamethoxazole and its N4-acetyl metabolite in biological fluids by high-pressure liquid chromatography. *J.High Resolut.Chromatogr.Chromatogr.Comm.*, **1980**, 3, 261–264
- Ferry, D.G.; McQueen, E.G.; Hearn, M.T.W. Sulfamethoxazole and trimethoprim estimation by high performance liquid chromatography. *Proc.Univ.Otago Med.Sch.*, **1978**, 56, 46–48

Sumatriptan

Molecular formula: C₁₄H₂₁N₃O₂S

Molecular weight: 295.4

CAS Registry No.: 103628-46-2 (sumatriptan), 103628-48-4 (sumatriptan succinate)



SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C2 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of water. 1 mL Plasma + 100 μ L 100 ng/mL IS in water, vortex briefly, add to the SPE cartridge at 2 mL/min, wash with 1 mL water, wash with two 1 mL portions of MeOH:water 30:70, elute with 1 mL MeOH:10 mM pH 5.0 ammonium acetate 60:40, evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 200 μ L mobile phase, sonicate for 10 min, vortex, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m CN (Beckman)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 36:6:58:0.1

Flow rate: 1.2

Injection volume: 25

Detector: MS, Sciex Model API III triple quadrupole, nebulizer probe 500°, nebulizing gas 80 psi, auxiliary flow 2 L/min, corona discharge needle +3 μ A, 0.1143 mm orifice, orifice 40 V, collision gas argon

CHROMATOGRAM

Retention time: 3

Internal standard: 3-[2-(diethylamino)ethyl]-N-methyl-1H-indole-5-methanesulfonamide (L-737,404, Merck) (3.35)

Limit of quantitation: 0.5 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

McLoughlin, D.A.; Olah, T.V.; Ellis, J.D.; Gilbert, J.D.; Halpin, R.A. Quantitation of the 5HT_{1D} agonists MK-462 and sumatriptan in plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J.Chromatogr.A*, **1996**, 726, 115-124

SAMPLE

Matrix: blood

Sample preparation: Condition an AASP C2 SPE cartridge (Jones Chromatography) with 1.8 mL MeOH and 1.8 mL water. Centrifuge plasma, remove a 1 mL aliquot and add it to 50 ng IS, vortex, add to the SPE cartridge, wash with 1.8 mL water, wash with 1.8 mL MeOH:water 30:70, elute the contents of the SPE cartridge onto the analytical column with the mobile phase.

HPLC VARIABLES**Guard column:** C18 Guard-Pak**Column:** 50 × 4.6 3 μm Spherisorb ODS-2**Mobile phase:** MeOH:100 mM ammonium acetate 60:40**Flow rate:** 1**Detector:** MS, thermospray, interface tip 148-152°, ion source 250°, m/z 296

CHROMATOGRAM**Retention time:** 1.2**Internal standard:** tritium-labeled sumatriptan (on N-methyl carbon) (m/z 299)**Limit of quantitation:** 2 ng/mL

KEY WORDS

pharmacokinetics; SPE; plasma

REFERENCE

Oxford, J.; Lant, M.S. Development and validation of a liquid chromatographic-mass spectrometric assay for the determination of sumatriptan in plasma. *J.Chromatogr.*, **1989**, 496, 137-146

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 1 mL Plasma + 100 μL 4 M NaOH + 2.6 mL ethyl acetate:dichloromethane 80:20, agitate on a rotational shaker for 15 min, centrifuge at 1000 g for 4 min. Remove 2 mL of the organic phase and add it to 300 μL pH 7 phosphate buffer and 2 mL hexane, agitate on a rotational shaker for 15 min, centrifuge at 1000 g for 4 min, freeze in acetone/dry ice, discard the organic layer, thaw the aqueous layer and inject a 200 μL aliquot. Urine. Dilute 20-fold with pH 7 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES**Guard column:** 10 mm long 5 μm Spherisorb ODS-1**Column:** 125 × 4.6 5 μm Spherisorb ODS-1**Mobile phase:** MeOH:buffer 60:40 (Buffer was 5.25 g Na₂HPO₄·2H₂O and 2.79 g KH₂PO₄ in 1 L water, pH 7.0.)**Column temperature:** 40**Flow rate:** 1**Injection volume:** 20-200**Detector:** E, ESA Coulochem model 5100A, 5011 analytical cell +0.55 V (conditioning cell) +0.8 V (analytical cell), guard cell +0.9 V; F ex 280 em 350 (see Pharm. Res. 1995, 12, 138)

CHROMATOGRAM**Limit of quantitation:** 1 ng/mL (plasma); 200 ng/mL (urine)

KEY WORDS

plasma; dog; rat; human; pharmacokinetics

REFERENCE

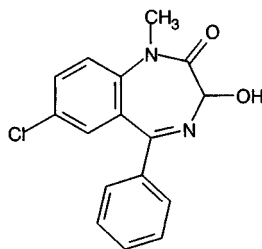
Andrew, P.D.; Birch, H.L.; Phillpot, D.A. Determination of sumatriptan succinate in plasma and urine by high-performance liquid chromatography with electrochemical detection. *J.Pharm.Sci.*, **1993**, 82, 73-76

Temazepam

Molecular formula: C₁₆H₁₃ClN₂O₂

Molecular weight: 300.7

CAS Registry No.: 846-50-4



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 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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: 4.58

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amoxapine, aspirin, astemizole, atenolol, benazepril, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, levomepromazine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mephenesin, mephentermine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phencyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin,

prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, sotalol, strychnine, sulfinpyrazole, sulindac, sulpride, sultopride, suriclone, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, tiocolmarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, vinblastine, vincristine, vandesine, warfarin, zopiclone, zorubicine

Interfering: alminoprofen, amodiaquine, benperidol, chloroquine, cicletanine, cocaine, doxylamine, droperidol, hydroxychloroquine, ketoprofen, labetalol, lidocaine, meperidine, mepivacaine, moclobemide, secobarbital, timolol, viloxazine, yohimbine, zolpidem

KEY WORDS

whole blood; plasma

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: 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 the SPE cartridge, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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: 5.3

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, 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, meth-

amphetamine, 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, maprotiline, methadone, nordiazepam, norfluoxetine, propoxyphene

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: Inject 100-200 μ L 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 \times 4 12 μ m TSK-gel G 3 PW (Tosohass); B 75 \times 4.6 Ultrasphere ODS C18 3 μ m

Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. X was MeCN. Y was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. X:Y 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: 23.5

OTHER SUBSTANCES

Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, tetrazepam, tofisopam, triazolam

Noninterfering: carbamazepine, ethosuximide, phenobarbital, phenytoin, 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: Filter (0.5 μm) serum, inject 200 μL directly onto column A with mobile phase A, run with mobile phase A for 1.5 min then change to mobile phase B over 0.1 min, wash column A with mobile phase B for 10.5 min, backflush column A onto column B with mobile phase C for 7.5 min then switch column B out of circuit, elute column B with mobile phase C and monitor the eluant, re-equilibrate column A with mobile phase A for at least 5 min.

HPLC VARIABLES

Column: A 15 \times 3.2 5 μm Brownlee ODS; B 250 \times 1 5 μm Adsorbosphere ODS
Mobile phase: A 10 mM sodium dodecyl sulfate; B water; C MeOH:water 65:35
Flow rate: A 1; B 1; C 0.06
Injection volume: 200
Detector: UV 242

CHROMATOGRAM

Retention time: 26
Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: diazepam, nordiazepam, oxazepam

KEY WORDS

serum; column-switching; microbore

REFERENCE

Koenigbauer, M.J.; Curtis, M.A. Use of micellar mobile phases and microbore column switching for the assay of drugs in physiological fluids. *J.Chromatogr.*, **1988**, *427*, 277–285

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 2 mL water + 50 μL 3.2 $\mu\text{g}/\text{mL}$ 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 \times 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: 6
Internal standard: estazolam (4)
Limit of detection: 8 ng/mL

OTHER SUBSTANCES

Extracted: diazepam, nordiazepam, oxazepam, triazolam
Simultaneous: bromazepam, flunitrazepam, nitrazepam, sulpride
Noninterfering: haloperidol, trihexyphenidyl
Interfering: clorazepate

KEY WORDS

serum; pharmacokinetics

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, dialysate, urine

Sample preparation: Condition a 100 mg C18 Bond Elut SPE cartridge with 2 mL MeOH then 2 mL water. 1 mL Plasma or 1 mL urine or 10 mL dialysate + 1 mL MeCN:water 30:70, vortex for 10 s, centrifuge at 4000 g for 5 min, add to the SPE cartridge, wash with 2 mL MeCN:water 20:80, dry for 3 to 4 min, elute with four 200 μ L aliquots of MeOH, evaporate combined eluates under nitrogen and take up residue in 100 μ L MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 Lichrocart Lichrosorb RP 18-5

Column: 5 μ m Lichrocart 125-4 Lichrospher 100 RP 18 endcapped

Mobile phase: MeCN:10 mM pH 5.6 buffer 40:60 (Prepare 1 M buffer from 94.8 mL 1 M KH_2PO_4 + 5.2 mL 1 M K_2HPO_4 , dilute to 10 mM with water.)

Flow rate: 1.6

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 3.17

Internal standard: climazolam (5.62)

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: oxazepam

KEY WORDS

plasma; SPE

REFERENCE

Chopineau, J.; Rivault, F.; Sautou, V.; Sommier, M.F. Determination of temazepam and its active metabolite, oxazepam in plasma, urine and dialysate using solid-phase extraction followed by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1994**, *17*, 373–383

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: Tissue homogenates were 1:2 in water. 1 mL Sample + 1 mL saturated sodium borate buffer + 100 μ L 20 μ g/mL methyl clonazepam in water + 5 mL n-butyl chloride, rotate at 40 rpm for 30 min, centrifuge at 2500 rcf for 5 min. Remove the organic phase and evaporate it to dryness at 70° under a stream of air, reconstitute the residue in 300 μ L mobile phase, vortex for 30 s, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 2.1 Analytichem ODS with an integral guard column

Mobile phase: MeCN:100 mM KH_2PO_4 300:700, adjust pH to 3.00 with concentrated phosphoric acid

Column temperature: 60

Flow rate: 1.5

Injection volume: 20

Detector: UV 242

CHROMATOGRAM**Retention time:** 4.57**Internal standard:** methyl clonazepam (5.36)**Limit of detection:** 50 ng/mL

OTHER SUBSTANCES**Extracted:** diazepam, trazodone**Also analyzed:** acetaminophen, alprazolam, amitriptyline, amoxapine, carbamazepine, chlordiazepoxide, chlorpromazine, chlorprothixene, clonazepam, demoxepam, desipramine, diphenhydramine, disopyramide, doxepin, ethotoin, flurazepam, glutethimide, haloperidol, haloperidol, imipramine, lidocaine, lorazepam, loxapine, maprotiline, mesantoin, mesoridazine, methaqualone, methotrimeprazine, nordiazepam, nortriptyline, oxazepam, pentazocine, perphenazine, phenacetin, phenobarbital, phenytoin, promazine, promethazine, propranolol, protriptyline, salicylic acid, thiothixene, trifluoperazine, trifluopromazine, trimipramine**Noninterfering:** chloral hydrate, codeine, ketamine, meperidine, methadone, methamphetamine, methyprylon, thioridazine

KEY WORDSserum; plasma; whole blood

REFERENCERoot, I.; Ohlson, G.B. Trazodone overdose: report of two cases. *J.Anal.Toxicol.*, **1984**, *8*, 91-94

SAMPLE**Matrix:** blood, milk**Sample preparation:** 500 μ L Plasma or milk + 25 μ L 5 μ g/mL flurazepam in water: MeCN 2.5:97.5 + 500 μ L 67 mM pH 7.4 phosphate buffer + 7 mL diethyl ether, extract for 15 min (A). Remove ether layer and add it to 1 mL 1.5 M HCl, shake for 15 min. Freeze and discard ether phase. Basify aqueous phase with 1 mL 2 M NaOH, extract with 7 mL diethyl ether for 15 min. Evaporate ether at 37° under a stream of nitrogen and take up residue in mobile phase, inject an aliquot. (For plasma **only** ether at (A) can be evaporated at 37° under a stream of nitrogen, take up residue in mobile phase, inject an aliquot.)

HPLC VARIABLES**Guard column:** 25 \times 4 5 μ m LiChrospher 60 RP-select B**Column:** 125 \times 4 5 μ m LiChrospher 60 RP-select B**Mobile phase:** MeCN: 10 mM KH₂PO₄ 31:69, adjusted to pH 2.80 with phosphoric acid**Column temperature:** 45**Flow rate:** 2**Injection volume:** 50**Detector:** UV 241

CHROMATOGRAM**Retention time:** 6.3**Internal standard:** flurazepam (3.0)

OTHER SUBSTANCES**Extracted:** diazepam, nordiazepam, oxazepam

KEY WORDSplasma; human; rabbit

REFERENCEStebler, T.; Guentert, T.W. Determination of diazepam and nordiazepam in milk and plasma in the presence of oxazepam and temazepam. *J.Chromatogr.*, **1991**, *564*, 330-337

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: 1 mL Blood, urine, or liver homogenate + 1 mL 1.15 M pH 6.4 phosphate buffer, add 25 µg prazepam, extract with 5 mL n-butyl chloride, centrifuge. Remove the organic layer and evaporate it in a vortex-evaporator. Reconstitute the residue in 100 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil-10 ODS

Mobile phase: MeCN:1 mM pH 3.2 phosphate buffer 40:60

Column temperature: 50

Flow rate: 3

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 3.2

Internal standard: prazepam (7.2)

KEY WORDS

liver

REFERENCE

Martin, C.D.; Chan, S.C. Distribution of temazepam in body fluids and tissues in lethal overdose. *J.Anal.Toxicol.*, **1986**, *10*, 77–78

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: 1.1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Internal standard: prazepam

Limit of detection: 88 ng/mL

OTHER SUBSTANCES

Also analyzed: adinazolam, brotizolam, diazepam, midazolam, nordazepam, oxazepam

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: blood, urine

Sample preparation: 1 mL Serum or urine + 50 μ L 5 μ g/mL nitrazepam in MeOH + 4 mL n-butyl chloride, vortex for 15 s, centrifuge at 9000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute residue in 30 μ L mobile phase, inject a 25 μ L aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m Supelguard LC-8

Column: 150 \times 4.6 5 μ m Supelcosil LC-8

Mobile phase: MeOH:water:buffer 55:25:20 (Buffer was 2.78 g K_2HPO_4 + 25.04 g KH_2PO_4 in 4 L water.)

Flow rate: 2.2

Injection volume: 25

Detector: UV 230

CHROMATOGRAM

Retention time: 5.31

Internal standard: nitrazepam (3.19)

Limit of detection: 15.5 ng/mL

Limit of quantitation: 46.5 ng/mL

OTHER SUBSTANCES

Simultaneous: alprazolam, chlordiazepoxide, clonazepam, N-desalkylflurazepam, N-des-methyl diazepam, diazepam, flurazepam, lorazepam, midazolam, oxazepam, triazolam

KEY WORDS

serum

REFERENCE

Kunsmann, G.W.; Manno, J.E.; Przekop, M.A.; Manno, B.R.; Llorens, K.A.; Kunsmann, C.M. Determination of temazepam and temazepam glucuronide by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1991**, *568*, 427-436

SAMPLE

Matrix: formulations

Sample preparation: Weigh capsule contents, dissolve in 50 mL MeOH, add 5 mL 0.75 mg/mL sulfanilamide in MeOH, make up to 100 mL with MeOH, filter (0.45 μ m), inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 50 \times 4.6 5 μ m Supelcosil LC8DB C8

Mobile phase: MeOH:1% acetic acid 40:60

Flow rate: 1.5

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 2.8

Internal standard: sulfanilamide (0.8)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

capsules; stability-indicating

REFERENCE

Fatmi, A.A.; Hickson, E.A. Determination of temazepam and related compounds in capsules by high-performance liquid chromatography. *J.Pharm.Sci.*, **1988**, *77*, 87-89

SAMPLE

Matrix: formulations

Sample preparation: Dissolve contents of capsules in MeOH to give a final concentration of 0.1 mg/mL, filter (0.45 μ m), inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 LiChrosorb 10 RP-18

Mobile phase: MeOH:water 60:40

Flow rate: 3.5

Injection volume: 20

Detector: UV 258

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Simultaneous: degradation products, diazepam

KEY WORDS

capsules

REFERENCE

Gordon, S.M.; Freeston, L.K.; Collins, A.J. Determination of temazepam and its major degradation products in soft gelatin capsules by isocratic reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1986**, *368*, 180-183

SAMPLE

Matrix: hair

Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL MeOH, heat at 55° for 18 h, adjust pH to 9.5-10. 1 mL Extract + 1 μ g 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 μ L 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m Newguard RP-18

Column: 100 \times 4.6 Spheri-5 RP-C18

Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH₂PO₄ + 30 mL diethylamine.)

Flow rate: 2

Injection volume: 30

Detector: UV 214

CHROMATOGRAM

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, diazepam, dothiepin, flunitrazepam, haloperidol, imipramine, nitrazepam, nortriptyline, oxazepam

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: 1 mL Microsomal incubation + 5 mL dichloromethane, extract, evaporate to dryness, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Zorbax SB-C18

Mobile phase: MeCN:MeOH:water 10:40:50

Flow rate: 1

Detector: UV 232

CHROMATOGRAM

Internal standard: 6β-hydroxyprogesterone

OTHER SUBSTANCES

Extracted: diazepam

KEY WORDS

rat; human; liver

REFERENCE

Gelboin, H.V.; Krausz, K.W.; Goldfarb, I.; Buters, J.T.M.; Yang, S.K.; Gonzalez, F.J.; Korzekwa, K.R.; Shou, M. Inhibitory and non-inhibitory monoclonal antibodies to human cytochrome P450 3A3/4. *Biochem.Pharmacol.*, **1995**, *50*, 1841–1850

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: 8.86 (A), 6.70 (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, methyl dopa, 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, sulfapyrazone, sulindac, 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

some 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 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica

Mobile phase: MeCN:water 40:60

Flow rate: 1

Injection volume: 1000

Detector: UV 254

CHROMATOGRAM

Retention time: k' 5.92

KEY WORDS

chiral; $\alpha = 1.17$

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: solutions

Sample preparation: Dissolve the compound, S-trolox methyl ether (Fluka), dicyclohexylcarbodiimide, and 4-dimethylaminopyridine in dichloromethane, stir at room temperature for 1 h, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 300 × 0.32 5 μm LiChrosorb Diol
Mobile phase: Carbon dioxide:MeOH 91.5:8.5
Column temperature: 80
Injection volume: 0.2
Detector: UV 254

CHROMATOGRAM

Retention time: 64.2 (second peak)

KEY WORDS

derivatization; subcritical fluid chromatography; chiral; density of mobile phase 0.51 g/mL; resolution (R_s) 1.1

REFERENCE

Almquist, S.R.; Petersson, P.; Walther, W.; Markides, K.E. Direct and indirect approaches to enantiomeric separation of benzodiazepines using micro column techniques. *J.Chromatogr.A*, **1994**, 679, 139-146

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 226; UV 308

CHROMATOGRAM

Retention time: 8.6
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, 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 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, bromazepam, 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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclufenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, 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, phen-suximide, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, 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.

HPLC VARIABLES

Column: 250 × 4.6 Vydac C18

Mobile phase: MeCN:20 mM pH 7.0 phosphate buffer 55:45

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 4

Internal standard: prazepam (7)

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Yang, S.K. Acid-catalyzed ethanolysis of temazepam in anhydrous and aqueous ethanol solutions. *J.Pharm.Sci.*, **1994**, 83, 898–902

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 100 µL 5 mM pH 5.5 acetate buffer + 25 µL β-glucuronidase/arylsulfatase (0.235/0.065 U, Calbiochem), mix, heat at 37° for 16 h, add 50 µL 5-50 µg/mL prazepam in MeOH, add 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove a 2 mL aliquot of the organic layer and add it to 2 mL hexane and 2 mL 6 M HCl, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove 1 mL of the aqueous phase and adjust pH to 6 with 1 mL 6 M NaOH and 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150 µL mobile phase, inject a 60 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm LiChrospher 100 RP-18(e)

Mobile phase: MeOH:water:triethylamine 30:70:0.1, adjusted to pH 5.5 with phosphoric acid

Flow rate: 0.7

Injection volume: 60

Detector: UV 240

CHROMATOGRAM

Retention time: 7.7

Internal standard: prazepam (17.0)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: desmethyldiazepam, diazepam, oxazepam

Simultaneous: amitriptyline, caffeine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, flunitrazepam, flurazepam, haloperidol, imipramine, levomepromazine, mianserin, nitrazepam, nortriptyline, perphenazine, phenobarbital, phenytoin, sulpride, thioridazine, triazolam

Interfering: maprotiline

REFERENCE

Chiba, K.; Horii, H.; Chiba, T.; Kato, Y.; Hirano, T.; Ishizaki, T. Development and preliminary application of high-performance liquid chromatographic assay of urinary metabolites of diazepam in humans. *J.Chromatogr.B*, **1995**, 668, 77–84

SAMPLE**Matrix:** urine**Sample preparation:** Heat 5 mL urine + 1 mL temazepam in MeOH with 1 mL β -glucuronidase at 37° for 2.5 h, cool, adjust to pH 8.5 with saturated Na_2CO_3 , extract with 10 mL dichloromethane. Evaporate, take up the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 Brownlee 5 μm RP-8**Mobile phase:** MeCN: 10 mM KH_2PO_4 : n-nonylamine 450:550:0.6, adjusted to pH 3.2 with phosphoric acid**Flow rate:** 1.6**Detector:** UV 225

CHROMATOGRAM**Retention time:** 7**Internal standard:** temazepam

OTHER SUBSTANCES**Extracted:** alprazolam, triazolam

KEY WORDS

temazepam is IS

REFERENCEFraser, A.D. Urinary screening for alprazolam, triazolam, and their metabolites with the EMIT d.a.u. benzodiazepine metabolite assay. *J.Anal.Toxicol.*, **1987**, *11*, 263–266

ANNOTATED BIBLIOGRAPHYHaginaka, J.; Kanasugi, N. Enantioselectivity of bovine serum albumin-bonded columns produced with isolated protein fragments. *J.Chromatogr.A*, **1995**, *694*, 71–80 [chiral; also benzoin, clorazepate, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, lorazepam, lormetazepam, oxazepam, pranoprofen, warfarin]Benhamou-Batut, F.; Demotes-Mainard, F.; Labat, L.; Vinçon, G.; Bannwarth, B. Determination of flunitrazepam by liquid chromatography. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 931–936 [α -hydroxytriazolam (IS); extracted alprazolam, bromazepam, clobazam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, nordiazepam, oxazepam, triazolam]Yang, S.K. Base-catalysed rearrangement of temazepam. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 209–219 [simultaneous degradation products]Yang, T.J.; Pu, Q.L.; Yang, S.K. Hydrolysis of temazepam in simulated gastric fluid and its pharmacological consequence. *J.Pharm.Sci.*, **1994**, *83*, 1543–1547 [simultaneous degradation products]Fernández, P.; Hermida, I.; Bermejo, A.M.; López-Rivadulla, M.; Cruz, A.; Concheiro, L. Simultaneous determination of diazepam and its metabolites in plasma by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1991**, *14*, 2587–2599 [extracted diazepam, nordiazepam, oxazepam; carbamazepine (IS); SPE]Vree, T.B.; Baars, A.M.; Wuis, E.W. Direct high pressure liquid chromatographic analysis and preliminary pharmacokinetics of enantiomers of oxazepam and temazepam with their corresponding glucuronide conjugates. *Pharm.Weekbl.[Sci.]*, **1991**, *13*, 83–90Noggle, F.T., Jr.; Clark, C.R.; DeRuiter, J. Liquid chromatographic separation of some common benzodiazepines and their metabolites. *J.Liq.Chromatogr.*, **1990**, *13*, 4005–4021 [also alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, demoxepam, diazepam, estazolam, fludiazepam, flunitrazepam, flurazepam, halazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, prazepam, triazolam]

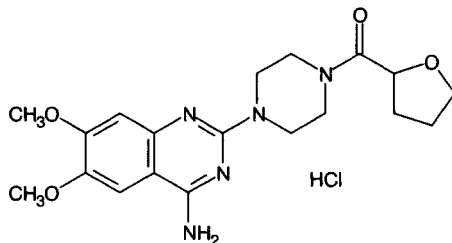
- Bastos, M.L.A. Improvement of HPLC conditions for separation of diazepam and its metabolites in biological extracts. *J.Liq.Chromatogr.*, **1989**, *12*, 1919–1934 [liver; brain; bile; kidney; prazepam (IS); extracted demethyl diazepam, diazepam, oxazepam; gradient]
- Pietrogrande, M.C.; Dondi, F.; Blo, G.; Borea, P.A.; Bigli, C. Retention behavior of benzodiazepines in normal-phase HPLC. Silica, cyano, and amino phases comparison. *J.Liq.Chromatogr.*, **1988**, *11*, 1313–1333 [also diazepam, lorazepam, medazepam, methyllorazepam, oxazepam, prazepam]
- Blaschke, G. Chromatographic resolution of chiral drugs on polyamides and cellulose triacetate. *J.Liq.Chromatogr.*, **1986**, *9*, 341–368 [also camazepam, chlormezanone, chlorthalidone, ifosfamide, ketamine, methaqualone, mianserin, oxapadol, oxazepam, oxazolam, praziquantel, rolipram]
- Pietrogrande, M.C.; Bigli, C.; Borea, P.A.; Barbaro, A.M.; Guerra, M.C.; Biagi, G.L. Relationship between $\log k'$ values of benzodiazepines and composition of the mobile phase. *J.Liq.Chromatogr.*, **1985**, *8*, 1711–1729 [also carbenicillin, chlordiazepoxide, diazepam, dicloxacillin, flurazepam, lorazepam, medazepam, methyllorazepam, nitrazepam, oxazepam, prazepam, testosterone]
- Ho, P.C.; Triggs, E.J.; Heazlewood, V.; Bourne, D.W. Determination of nitrazepam and temazepam in plasma by high-performance liquid chromatography. *Ther.Drug Monit.*, **1983**, *5*, 303–307

Terazosin

Molecular formula: C₁₉H₂₅N₅O₄

Molecular weight: 387.4

CAS Registry No.: 65390-64-7 (terazosin),
70024-40-7 (terazosin hydrochloride dihydrate),
63074-08-8 (terazosin hydrochloride)



SAMPLE

Matrix: blood, dialysate, urine

Sample preparation: 200 μ L Serum, plasma, urine, or dialysate + 400 μ L 200 ng/mL prazosin hydrochloride in MeCN at 5°, mix, centrifuge at 5° for 5-10 min. Remove the supernatant and evaporate it to dryness at 40-50° under a stream of air. Dissolve residue in 400 μ L 100 mM mobile phase, inject a 50-100 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 5 μ m Spherisorb ODS

Mobile phase: MeCN:THF:4-20 mM pH 5 sodium phosphate 25:6:69

Flow rate: 1.6

Injection volume: 50-100

Detector: F ex 250 em 370 (filter)

CHROMATOGRAM

Retention time: 6

Internal standard: prazosin (9)

Limit of quantitation: 5 ng/mL (blood, dialysate), 50 ng/mL (urine)

KEY WORDS

serum; plasma; human; dog

REFERENCE

Patterson, S.E. Rapid and sensitive analysis of terazosin in plasma, peritoneal dialysis solution, and urine using high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, 1984, 311, 206-212

SAMPLE

Matrix: blood, dialysate, urine

Sample preparation: 1 mL Plasma, 1 mL dialysate, or 200 μ L urine + 100 μ L 1 M NaOH, mix, add 5 mL ethyl acetate:benzene 20:80 containing 2-50 ng/mL prazosin hydrochloride (Caution! Benzene is a carcinogen!), vortex for 5 min, centrifuge. Remove 4-4.5 mL of the organic phase and evaporate it to dryness at 40-50° under a stream of air. Dissolve residue in 300 μ L 100 mM phosphoric acid or mobile phase, inject a 15-100 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m IBM C 1

Mobile phase: MeCN:THF:4-20 mM pH 7.0 sodium phosphate 22:6:72

Flow rate: 1

Injection volume: 15-100

Detector: F ex 250 em 370 (filter)

CHROMATOGRAM

Retention time: 5

Internal standard: prazosin (8)

Limit of quantitation: 1 ng/mL (blood, dialysate), 10 ng/mL (urine)

KEY WORDS

plasma; human; dog

REFERENCE

Patterson, S.E. Rapid and sensitive analysis of terazosin in plasma, peritoneal dialysis solution, and urine using high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, 1984, 311, 206-212

SAMPLE

Matrix: blood, urine

Sample preparation: 50 μ L Plasma or urine + 100 μ L saturated NaCl + 50 μ L 2 μ g/mL dimethothiazine mesylate in water + 50 μ L 4 M NaOH, vortex for 10 s, add 200 μ L MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 110 μ L aliquot of the organic phase.

HPLC VARIABLES

Column: 250 \times 5 μ m Spherisorb S5W

Mobile phase: MeOH:10 mM ammonium perchlorate adjusted to pH 6.7 with 1 mL/L methanolic NaOH (0.1 M)

Flow rate: 2

Injection volume: 110

Detector: F ex 370 em 370-700

CHROMATOGRAM

Retention time: 4

Internal standard: dimethothiazine mesylate (5)

Limit of detection: 1 μ g/mL

OTHER SUBSTANCES

Extracted: prazosin

Simultaneous: N-acetylprocainamide, ajmaline, chlorpromazine, desipramine, dipyridamole, doxazosin, flecainide, flurazepam, gallopamil, imipramine, ketanserin, metoprolol, mexiletine, mianserin, nadolol, nitrazepam, orphenadrine, oxprenolol, penbutolol, pindolol, prajmalium, procainamide, propranolol, protriptyline, pyrimethamine, quinidine, quinine, triamterene, trimipramine, verapamil

Noninterfering: amiodarone, atenolol, disopyramide, labetalol, lignocaine, lorcaïnide, methyl dopa, nifedipine, prenalterol, propafenone, sotalol, timolol

KEY WORDS

plasma

REFERENCE

Bhamra, R.K.; Flanagan, R.J.; Holt, D.W. High-performance liquid chromatographic measurement of prazosin and terazosin in biological fluids. *J.Chromatogr.*, 1986, 380, 216-221

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 100 mg bulk material in 100 mL MeCN:50 mM pH 4.4 citrate buffer containing 10 mL 0.2 mg/mL 3,4-dimethoxybenzoic acid in MeOH, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 μ m Zorbax Rx C8

Mobile phase: MeCN:isopropanol:50 mM pH 4.4 citrate buffer 175:50:1775

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 27

Internal standard: 3,4-dimethoxybenzoic acid

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

rugged

REFERENCE

Bauer, J.F.; Krogh, S.K.; Chang, Z.L.; Wong, C.F. Determination of minor impurities in terazosin hydrochloride by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, 648, 175-181

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, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, dipyrindamole, 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, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phenotolamine, 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, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

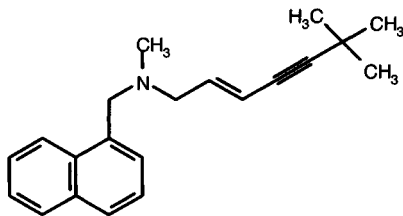
- Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection. *J.Chromatogr.*, **1985**, *323*, 191–225

Terbinafine

Molecular formula: C₂₁H₂₅N

Molecular weight: 291.4

CAS Registry No.: 91161-71-6



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 100 μ L 5 μ g/mL IS in water + 1 mL 200 mM pH 9 borate buffer + 8 mL n-hexane, shake horizontally at 200 rpm for 25 min, centrifuge at 2000 g for 10 min. Remove 7 mL of the supernatant and add it to 1 mL 500 mM sulfuric acid:isopropanol 85:15, shake for 15 min, centrifuge at 2000 g for 5 min, inject a 250 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 100 \times 4.6 3 μ m Pecospher C18

Mobile phase: MeCN:buffer 50:50 (Buffer was 3.35 mL triethylamine and 2.65 mL orthophosphoric acid in 2 L water.)

Flow rate: 1

Injection volume: 250

Detector: UV 224

CHROMATOGRAM

Retention time: 9.9

Internal standard: 2-(1-naphthyl)-1-(3-phenylprop-2-enyl)piperidine hydrochloride (IW 85-190) (12.4)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Denouël, J.; Keller, H.P.; Schaub, P.; Delaborde, C.; Humbert, H. Determination of terbinafine and its desmethyl metabolite in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 663, 353-359

SAMPLE

Matrix: blood

Sample preparation: Add IS to plasma, adjust pH to 9, extract with n-hexane. Add the organic layer to 500 mM sulfuric acid:isopropanol 85:15, extract, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 100 \times 4.6 Spherisorb RP-18/5

Mobile phase: MeCN:10 mM K₂HPO₄:triethylamine 60:40:0.01

Column temperature: 50

Flow rate: 1

Detector: UV 224

CHROMATOGRAM

Internal standard: 2-(1-naphthyl)-1-(3-phenylprop-2-enyl)piperidine hydrochloride (SDZ 85-190)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kovarik, J.M.; Kirkesseli, S.; Humbert, H.; Grass, P.; Kutz, K. Dose-proportional pharmacokinetics of terbinafine and its N-demethylated metabolite in healthy volunteers. *Br.J.Dermatol.*, **1992**, *126 Suppl 39*, 8-13

SAMPLE

Matrix: blood, milk

Sample preparation: Plasma. 100 μ L Plasma + 300 μ L 4 g/L ammonium acetate in MeOH, centrifuge, inject a 200 μ L aliquot of the supernatant. Milk. 100 μ L Milk + 100 μ L 100 mM NaCl (?) + 800 μ L MeCN, centrifuge, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m RP-8 (Merck)

Mobile phase: MeOH:10 mM pH 7 phosphate buffer 77:23 containing 4 g/L ammonium acetate

Column temperature: 35

Flow rate: 1.5

Injection volume: 100-200

Detector: E, Metrohm 656, glassy carbon electrode + 1.1 V

CHROMATOGRAM

Retention time: 10.50 (plasma), 12.03 (milk)

Limit of detection: 50 ng/mL (plasma); 150 ng/mL (milk)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; human; rat; dog

REFERENCE

Schatz, F.; Haberl, H. Analytical methods for the determination of terbinafine and its metabolites in human plasma, milk and urine. *Arzneimittelforschung*, **1989**, *39*, 527-532

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 750 μ L Plasma + 50 μ L 320 μ g/mL IS + 25 μ L 85% phosphoric acid + 750 μ L EtOH:isopropanol 75:25, vortex for 10 s, let stand on crushed ice for 30 min, centrifuge at 1000 g for 15 min. Remove a 400 μ L aliquot of the supernatant and add it to 400 μ L 10 mM pH 5 phosphate buffer, vortex for 10 s, inject a 250 μ L aliquot onto column A with mobile phase A and elute to waste. After 4 min backflush column A with mobile phase A to waste, after 1 min backflush the contents of column A onto column B with mobile phase B, after 10 min remove column A from the circuit, elute

column B with mobile phase B and monitor the effluent. (Recondition column A with mobile phase A for 30 min at 0.1 mL/min). Urine. 500 μ L Urine + 50 μ L 220 μ g/mL IS + 200 μ L 10000 IU/mL β -glucuronidase (bovine liver, Sigma) in 10 mL 10 mM pH 5 phosphate buffer, heat at 37° for 18 h, add 300 μ L 10 mM pH 5 phosphate buffer, vortex for 10 s, centrifuge at 2500 g for 18 min, inject a 100-250 μ L aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 4 min backflush column A with mobile phase A to waste, after 1 min backflush the contents of column A onto column B with mobile phase B, after 10 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent. (Recondition column A with mobile phase A for 30 min at 0.1 mL/min).

HPLC VARIABLES

Column: A 15 \times 3.2 30-40 μ m Perisorb RP-2 (Merck); B 220 \times 4.6 5 μ m Spheri-5 phenyl
Mobile phase: A 20 mM KH_2PO_4 containing 0.25% triethylamine, pH adjusted to 3.8 with 1 M phosphoric acid; B MeCN:buffer 55:45 (Buffer was 20 mM KH_2PO_4 containing 0.125% triethylamine, pH adjusted to 3.8 with 1 M phosphoric acid.)

Column temperature: 30-35

Flow rate: 1

Injection volume: 100-250

Detector: UV 224

CHROMATOGRAM

Retention time: 34-36

Internal standard: 2-(1-naphthyl)-1-(3-phenylprop-2-enyl)piperidine hydrochloride (IW 85-190)

Limit of quantitation: 20 ng/mL (plasma); 100 ng/mL (urine)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Zehender, H.; Denouël, J.; Roy, M.; Le Saux, L.; Schaub, P. Simultaneous determination of terbinafine (Lamisil) and five metabolites in human plasma and urine by high-performance liquid chromatography using on-line solid-phase extraction. *J. Chromatogr. B*, **1995**, 664, 347-355

SAMPLE

Matrix: nails

Sample preparation: 10-20 mg Nail clippings + 1 mL 1 M NaOH, let stand at room temperature for 48 h, adjust pH to 9 with 1 M HCl, adjust molarity to 0.2 with water, add 100 μ L 10 mg/mL proteinase K (Type I fungal proteinase, P0390, Sigma), heat at 37° for 12-16 h, add 50 ng IS, vortex, add 3 mL hexane, vortex for 3 min, add 100 μ L MeOH, centrifuge at 500 g for 10 min. Remove a 2.7 mL aliquot of the organic layer and add it to 200 μ L 500 mM sulfuric acid:propranolol 85:15, vortex for 3 min, centrifuge, inject a 100 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 20 \times 2 5-20 μ m Prepsil RPC8 (Apex)

Column: 250 mm long 5 μ m Spherisorb C18

Mobile phase: MeCN:10 mM phosphate buffer:triethylamine 80:20:0.1, pH adjusted to 4.0 with 85% phosphoric acid

Flow rate: 2

Injection volume: 100

Detector: UV 224

CHROMATOGRAM**Retention time:** 7.5**Internal standard:** 2-(1-naphthyl)-1-(3-phenylprop-2-enyl)piperidine hydrochloride (Sandoz 85-190) (8.4)

OTHER SUBSTANCES**Extracted:** metabolites

REFERENCE

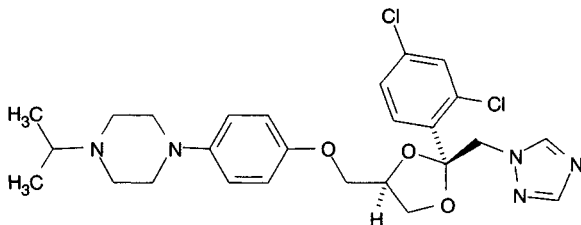
Dykes, P.J.; Thomas, R.; Finlay, A.Y. Determination of terbinafine in nail samples during systemic treatment for onychomycoses. *Br.J.Dermatol.*, **1990**, *123*, 481–486

Terconazole

Molecular formula: C₂₆H₃₁Cl₂N₅O₃

Molecular weight: 532.5

CAS Registry No.: 67915-31-5



SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond-Elut C18 cartridge with 6 mL MeOH and 6 mL water. 1 mL Serum + 250 µL 100 mM NaOH + 3 mL water, add to cartridge, wash with 9 mL water, wash with 200 µL MeOH, elute with 1 mL MeOH. Evaporate eluent at 60°, resuspend in 200 µL mobile phase, centrifuge at 13000 g for 2 min, inject 20-40 µL.

HPLC VARIABLES

Guard column: Chrompack C18

Column: 100 × 3 Hypersil ODS in a Chrompack glass cartridge

Mobile phase: MeCN:water 45:55 containing 500 µL/L diethylamine, pH adjusted to 8.0 with orthophosphoric acid

Flow rate: 0.6

Injection volume: 20-40

Detector: UV 254

CHROMATOGRAM

Retention time: 11.4

Internal standard: terconazole

OTHER SUBSTANCES

Extracted: diazepam, ketoconazole

Noninterfering: acetaminophen, acyclovir, allopurinol, amoxicillin, amphotericin B, ampicillin, aspirin, azlocillin, bendrofluazide, bumetanide, buprenorphine, carbenicillin, cefazolin, cefotaxime, cefoxitin, ceftazidime, cefuroxime, cephalexin, chlorambucil, chloramphenicol, chlordiazepoxide, chlorpheniramine, chlorpropamide, cyclophosphamide, cyclosporin, cytarabine, daunorubicin, dextropropoxyphene, dihydrocodeine, domperidone, flucytosine, furosemide, gentamicin, griseofulvin, melphalan, methotrexate, metochlopramide, metronidazole, miconazole, nabilone, netilmicin, nicotinamide, nitrazepam, penicillin G, piperacillin, prednisolone, procarbazine, prochlorperazine, riboflavin, rifampin, sulfamethoxazole, thioguanine, tobramycin, tolbutamide, trimethoprim

KEY WORDS

serum; terconazole is IS

REFERENCE

Turner, C.A.; Turner, A.; Warnock, D.W. High performance liquid chromatographic determination of ketoconazole in human serum. *J. Antimicrob. Chemother.*, **1986**, *18*, 757-763

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 4.5 µBondapak C18

Mobile phase: MeOH:buffer 60:40 (Buffer was 25 mM KH₂PO₄ + 4 mM heptanesulfonic acid, adjusted to pH 8.0 with 1 M NaOH.)

Flow rate: 1.8
Injection volume: 75
Detector: UV 226

OTHER SUBSTANCES

Simultaneous: ketoconazole

REFERENCE

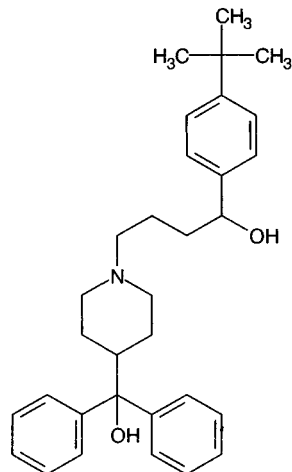
Carver, P.L.; Berardi, R.R.; Knapp, M.J.; Rider, J.M.; Kauffman, C.A.; Bradley, S.F.; Atassi, M. In vivo interaction of ketoconazole and sucralfate in healthy volunteers. *Antimicrob.Agents Chemother.*, **1994**, *38*, 326–329

Terfenadine

Molecular formula: C₃₂H₄₁NO₂

Molecular weight: 471.7

CAS Registry No.: 50679-08-8



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 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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: 18.33

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carboxamine, carpiramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorophenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyrindamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenfluramine, fenopropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, gliben-

clamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mexiletine, mianserine, midazolam, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozone, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfipyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, ticlopidine, timolol, tioclomarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, viloxazine, vinblastine, vincristine, vindesine, warfarin, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: bepridil, penfluridol

KEY WORDS

whole blood; plasma

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 + 500 μ L 100 mM HCl + 5 mL 50 ng/mL propranolol in MTBE:isopropanol 95:5, vortex for 20 s, centrifuge at 3000 g for 15 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, dissolve the residue in 100 μ L MeOH, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m Microsorb cyanopropylsilane

Mobile phase: MeCN:buffer 75:25 (Buffer was 1 mM sodium acetate adjusted to pH 4.0 with acetic acid.)

Injection volume: 40

Detector: F ex 230 em 300 (cut-off filter 270 nm)

CHROMATOGRAM

Retention time: 15.1

Internal standard: propranolol (12.2)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: acetaminophen, aspirin, ibuprofen, pseudoephedrine, tricyclic antidepressants

KEY WORDS

plasma

REFERENCE

Surapaneni, S.; Khalil, S.K.W. A sensitive HPLC method for the determination of terfenadine and its metabolite in human plasma. *J.Liq.Chromatogr.*, **1994**, *17*, 2419–2428

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 500 mg Analytichem SPE C18 cartridge with two 2 mL volumes of MeOH, 2 mL water, 1.5 mL 200 mM pH 4.0 acetate buffer. Add 1 mL 75 ng/mL IS in 200 mM pH 4.0 acetate buffer and 1 mL plasma to the SPE cartridge and blow through with nitrogen. Wash with 2 mL water, two 1 mL portions of MeOH:water 50:50, and 1 mL MeOH. Dry column by blowing nitrogen through at 8 mL/min for 5 min. Elute with two 500 μ L portions of 50 mM triethylamine in MeOH. Evaporate the eluate to dryness at 55° under a stream of nitrogen and dissolve the residue in 200 μ L mobile phase, inject a 150 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb S5CN cyano

Mobile phase: MeCN:MeOH:buffer 19:29:52 (Buffer was 12 mM, prepared from 0.5 g ammonium acetate and 5 mL acetic acid in 520 mL water.)

Column temperature: 35

Flow rate: 1.8

Injection volume: 150

Detector: F ex 230 em 280

CHROMATOGRAM

Retention time: 13.5

Internal standard: MDL 26,042 (9.3)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: acetaminophen, ephedrine, ibufenac, ibuprofen, norpseudoephedrine, pseudoephedrine

KEY WORDS

plasma; SPE

REFERENCE

Coutant, J.E.; Westmark, P.A.; Nardella, P.A.; Walter, S.M.; Okerholm, R.A. Determination of terfenadine and terfenadine acid metabolite in plasma using solid-phase extraction and high-performance liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1991**, *570*, 139–148

SAMPLE

Matrix: blood

Sample preparation: Add 1 mL plasma to an Analytichem C18 SPE cartridge, elute with MeOH:200 mM pH 4 sodium acetate 95:5, evaporate eluate to dryness under reduced pressure, reconstitute in 200 μ L mobile phase A, inject a 150 μ L aliquot onto column A with mobile phase A, collect terfenadine fraction at 12 min, evaporate to dryness, dissolve residue in 100 μ L mobile phase B, inject an 80 μ L aliquot onto column B with mobile phase B, monitor effluent from column B.

HPLC VARIABLES

Column: A 150 \times 4.6 Spherisorb CN; B 150 \times 4.6 5 μ m Ultron ES-OVM ovomucoid (MAC-MOD Analytical)

Mobile phase: A MeOH:MeCN:12 mM pH 4.0 sodium acetate 35:13:52; B MeOH:25 mM pH 4.6 phosphate buffer 25:75

Flow rate: A 1.5; B 1
Injection volume: A 150; B 80
Detector: F ex 200 em 280

CHROMATOGRAM

Retention time: 7 (S), 9 (R) (measured after injection on column B)

OTHER SUBSTANCES

Also analyzed: metabolites

KEY WORDS

plasma; SPE; rat; chiral

REFERENCE

Zamani, K.; Conner, D.P.; Weems, H.B.; Yang, S.K.; Cantilena, L.R., Jr. Enantiomeric analysis of terfenadine in rat plasma by HPLC. *Chirality*, **1991**, 3, 467-470

SAMPLE

Matrix: microsomal incubations

Sample preparation: 2 mL Microsomal incubation + 1.6 mL cold MeCN + 400 μ L 500 ng/mL IS in MeCN, centrifuge at 10000 g for 10 min. Add a 500 μ L aliquot of the supernatant to 400 μ L acetate buffer, inject a 200 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Spherisorb CN

Mobile phase: MeCN: 100 mM ammonium acetate buffer 40:60

Column temperature: 35

Flow rate: 1.3

Injection volume: 200

Detector: F ex 230 em 280

CHROMATOGRAM

Retention time: 10

Internal standard: MDL 16,232 (Marion Merrell Dow) (11.5)

OTHER SUBSTANCES

Extracted: metabolites, azacyclonol

KEY WORDS

human; liver

REFERENCE

Ling, K.-H.J.; Leeson, G.A.; Burmaster, S.D.; Hook, R.H.; Reith, M.K.; Cheng, L.K. Metabolism of terfenadine associated with CYP3A(4) activity in human hepatic microsomes. *Drug Metab. Dispos.*, **1995**, 23, 631-636

SAMPLE

Matrix: microsomal incubations

Sample preparation: Add an equal volume of chilled MeOH to 0.25-1 mL microsomal incubation, let stand on ice for 5 min, centrifuge at 2000 g for 10 min, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 5 μ m cyano guard column (YMC)

Column: 250 \times 4.6 5 μ m YMC-Pack cyano (YMC)

Mobile phase: Gradient. MeCN:10 mM pH 4.0 ammonium acetate 25:75 for 40 min, to 50:50 over 5 min

Column temperature: 35

Flow rate: 1

Injection volume: 50

Detector: UV 230; F ex 230 em 280; radioactivity

CHROMATOGRAM

Retention time: 55

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver; tritium labeled

REFERENCE

Rodrigues, A.D.; Mulford, D.J.; Lee, R.D.; Surber, B.W.; Kukulka, M.J.; Ferrero, J.L.; Thomas, S.B.; Shet, M.S.; Estabrook, R.W. *In vitro* metabolism of terfenadine by a purified recombinant fusion protein containing cytochrome P4503A4 and NADPH-P450 reductase. Comparison to human liver microsomes and precision-cut liver tissue slices. *Drug Metab.Dispos.*, **1995**, *23*, 765-775

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: 12.24

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, flvoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, flvoxamine, 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, mephentoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, na-

phazoline, 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, sulfapyrazone, sulindac, temazepam, terbutaline, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinamide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 × 4.6 5 μm Ultron ES-OVM (Grom)

Mobile phase: Gradient. Isopropanol: 100 mM pH 5.5 phosphate buffer 4:96 for 20 min, to 12:88 over 1 min, maintain at 12:88 for 19 min.

Flow rate: 1

Detector: UV 224; F ex 230 em 280

CHROMATOGRAM

Retention time: 28 (R-(+)), 34 (S(-))

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

chiral

REFERENCE

Terhechte, A.; Blaschke, G. Investigation of the stereoselective metabolism of the chiral H₁-antihistaminic drug terfenadine by high-performance liquid chromatography. *J.Chromatogr.A*, **1995**, *694*, 219–225

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:** 1.384

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methypylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine

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:** 150 × 4.6 5 μm MicroPak MCH-5**Mobile phase:** MeCN:30 mM pH 3.0 phosphate buffer 70:30**Flow rate:** 1.7**Injection volume:** 20**Detector:** UV 230

REFERENCE

Fernández Otero, G.C.; Lucangioli, S.E.; Carducci, C.N. Adsorption of drugs in high-performance liquid chromatography injector loops. *J.Chromatogr.A*, **1993**, *654*, 87–91

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Cyclobond I β-cyclodextrin (Advanced Separations Technologies)**Mobile phase:** Hexane:MeOH:EtOH 90:5:5**Flow rate:** 2**Detector:** UV 220

CHROMATOGRAM**Retention time:** 10.6 (S), 11.7 (R)

KEY WORDSchiral

REFERENCE

Weems, H.; Zamani, K. Resolution of terfenadine enantiomers by beta-cyclodextrin chiral stationary phase high-performance liquid chromatography. *Chirality*, **1992**, *4*, 268–272

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: 1.76

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phen-tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pin-dolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pir-tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, tri-fluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimetho-prim, trimipramine, tripeleppamine, 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 electro-chemical oxidation detection. *J.Chromatogr.*, **1985**, 323, 191–225

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 3 mL 100 mM sodium bicarbonate, extract twice with 5 mL ethyl acetate. Combine extracts and evaporate them to dryness at 55° under a stream of nitrogen. Dissolve the residue in 100 µL MeOH:water 20:80, inject a 10-30 µL aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Cyclobond I**Mobile phase:** MeOH:14 mM sodium perchlorate 75:25**Flow rate:** 0.2**Injection volume:** 10-30**Detector:** UV 210; UV 254

CHROMATOGRAM**Retention time:** 43 ((+)-R), 47 ((-)-S)

OTHER SUBSTANCES**Simultaneous:** metabolites

KEY WORDSchiral

REFERENCE

Chan, K.Y.; George, R.C.; Chen, T.-M.; Okerholm, R.A. Direct enantiomeric separation of terfenadine and its major acid metabolite by high-performance liquid chromatography, and the lack of stereoselective terfenadine enantiomer biotransformation in man. *J.Chromatogr.*, **1991**, *571*, 291–297

ANNOTATED BIBLIOGRAPHY

Jurima-Romet, M.; Crawford, K.; Cyr, T.; Inaba, T. Terfenadine metabolism in human liver. In vitro inhibition by macrolide antibiotics and azole antifungals. *Drug Metab.Dispos.*, **1994**, *22*, 849–857 [extracted metabolites; microsomal incubations; rat; column temp 35; LOQ 92 nM]

von Moltke, L.L.; Greenblatt, D.J.; Duan, S.X.; Harmatz, J.S.; Shader, R.I. In vitro prediction of the terfenadine-ketoconazole pharmacokinetic interaction. *J.Clin.Pharmacol.*, **1994**, *34*, 1222–1227 [clomipramine (IS); microsomal incubations]

Chen, T.M.; Chan, K.Y.; Coutant, J.E.; Okerholm, R.A. Determination of the metabolites of terfenadine in human urine by thermospray liquid chromatography-mass spectrometry. *J.Pharm.Biomed.Anal.*, **1991**, *9*, 929–933 [LC-MS; UV detection]

George, R.D.; Contario, J.J. Quantitation of terfenadine, pseudoephedrine hydrochloride, and ibuprofen in a liquid animal dosing formulation using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1988**, *11*, 475–488 [stability-indicating]

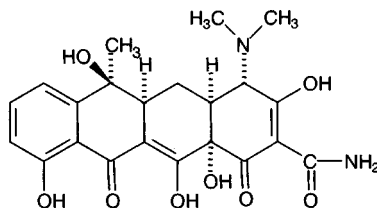
Gupta, S.K.; Gwilt, P.R.; Lim, J.K.; Waters, D.H. High-performance liquid chromatographic determination of terfenadine in commercial tablets. *J.Chromatogr.*, **1986**, *361*, 403–406

Tetracycline

Molecular formula: C₂₂H₂₄N₂O₈

Molecular weight: 444.4

CAS Registry No.: 60-54-8, 6416-04-2 (trihydrate),
64-75-5 (HCl), 1336-20-5 (phosphate)



SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 200 μ L 24% trichloroacetic acid in MeOH + 300 μ L mobile phase buffer (A), vortex for 1 min, centrifuge at 2000 g for 15 min, inject 50 μ L of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Capcell C18 type SG-120 (Shiseido)

Mobile phase: MeOH:buffer 60:40 (Buffer (A) was 100 mM pH 6.5 sodium acetate containing 35 mM calcium chloride and 25 mM disodium EDTA.)

Column temperature: 30 \pm 0.2

Flow rate: 1

Injection volume: 50

Detector: F ex 390 em 512

CHROMATOGRAM

Retention time: 8

Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Also analyzed: chlortetracycline, 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

Sample preparation: 250 μ L Plasma + 50 μ L 10 mM HCl + 1 mL buffer, vortex vigorously, add 6 mL ethyl acetate, add 500 μ L isopropanol, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 300 μ L mobile phase and 1 mL hexane, vortex, centrifuge at 9800 g for 5 min, inject a 100 μ L aliquot of the lower aqueous layer. (Buffer was 3 M NaH₂PO₄ containing 1 M sodium sulfite, pH ca. 5.4.)

HPLC VARIABLES

Column: 125 \times 4 5 μ m Lichrosorb RP 8

Mobile phase: MeCN:MeOH:10 mM oxalic acid 15:10:75

Flow rate: 1

Injection volume: 100

Detector: UV 357

CHROMATOGRAM**Retention time:** 4.5**Internal standard:** tetracycline

OTHER SUBSTANCES**Extracted:** oxytetracycline

KEY WORDS

plasma; cow; tetracycline is IS

REFERENCE

Nelis, H.J.; Vandenbranden, J.; De Kruif, A.; Belpaire, F.; De Leenheer, A.P. Liquid chromatographic determination of oxytetracycline in bovine plasma by double-phase extraction. *J.Pharm.Sci.*, **1992**, *81*, 1216-1218

SAMPLE**Matrix:** blood**Sample preparation:** 100 μ L Plasma + 20 μ L trifluoroacetic acid, mix 30 s in a whirl mixer, centrifuge at 5400 g for 5 min, inject supernatant (80 μ L).

HPLC VARIABLES**Guard column:** 10 μ m RP SAS-Hypersil**Column:** 125 \times 4.6 10 μ m RP SAS-Hypersil**Mobile phase:** MeCN: 10 mM phosphoric acid 18:82**Flow rate:** 2**Injection volume:** 80**Detector:** UV 365

CHROMATOGRAM**Retention time:** 2.1**Limit of detection:** 80 ng/mL

KEY WORDS

plasma

REFERENCE

Krämer-Horaczynska, F. High-performance liquid chromatographic procedures for the quantitative analysis of 15 tetracycline derivatives in small blood samples. *J.Chromatogr.Sci.*, **1991**, *29*, 107-113

SAMPLE**Matrix:** blood, urine**Sample preparation:** Serum. 500 μ L Serum + 50 μ L 6% ascorbic acid in water + 50 μ L 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 μ L 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μ L mobile phase, mix, filter, keep in ice, inject a 20 μ L aliquot. Urine. 100 μ L Urine + 50 μ L 6% ascorbic acid in water + 50 μ L demeclocycline in MeOH/100 mM HCl + 400 μ L 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 μ L 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μ L mobile phase, mix, filter, keep in ice, inject a 20 μ L aliquot. (Buffer was 27.6 g NaH₂PO₄ + 25.2 g sodium sulfite in 100 mL water, pH 6.1.)

HPLC VARIABLES**Column:** 100 \times 2.5 μ m Lichrosorb RP8

Mobile phase: MeCN:100 mM citric acid 24:76
Flow rate: 0.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM

Retention time: 3
Internal standard: demeclocycline (4)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, doxycycline, methacycline, oxytetracycline

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
Sample preparation: Prepare a 1 mg/mL solution, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 8 μ m PLRP-S 100 Å poly(styrene-divinylbenzene) (Polymer Laboratories)
Mobile phase: t-Butanol:3.5% pH 9.0 K₂HPO₄:1% pH 9.0 tetrabutylammonium sulfate:4% pH 9.0 sodium edetate:water 8.5:10:20:1:70.5 (w/v/v/v/v) (The solutions were adjusted to the required pH with 10% phosphoric acid and 8.5% NaOH.)
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

comparison of columns

REFERENCE

Hendrix, C.; Roets, E.; Crommen, J.; de Beer, J.; Porqueras, E.; Van den Bossche, W.; Hoogmartens, J. Collaborative study of the analysis of tetracycline by liquid chromatography on poly(styrene-divinylbenzene). *J.Liq.Chromatogr.*, **1993**, *16*, 3321–3329

SAMPLE

Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μ g/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 μ m), 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 20:80 (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)

Flow rate: 1

Detector: UV 280

CHROMATOGRAM

Retention time: 27

OTHER SUBSTANCES

Simultaneous: impurities, oxytetracycline

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: 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

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: impurities, chlortetracycline

REFERENCE

Muhammad, N.; Bodnar, J.A. Separation and quantitation of chlortetracycline, 4-epitetracycline, 4-epi-anhydrotetracycline, and anhydrotetracycline in tetracycline by high-performance liquid chromatography. *J.Pharm.Sci.*, 1980, 69, 928–930

SAMPLE

Matrix: cell suspensions

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 μ L mobile phase, inject 75 μ L.

HPLC VARIABLES

Column: μ Bondapak C18

Mobile phase: MeCN:50 mM pH 3.1 KH_2PO_4 45:55

Flow rate: 1

Injection volume: 75

Detector: UV 353

CHROMATOGRAM

Retention time: 2.4

Internal standard: minocycline

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: food

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 a 100 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m 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: 3.5

Limit of detection: 0.02 ppm

OTHER SUBSTANCES

Extracted: chlortetracycline, doxycycline, oxytetracycline

KEY WORDS

honey; SPE

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: food

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 μ L 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 μ L aliquot.

HPLC VARIABLES

Column: 75 \times 4.6 3 μ m 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

Limit of detection: 0.1 ppm

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline (demethylchlortetracycline), doxycycline, methacycline, minocycline, oxytetracycline

KEY WORDS

honey; 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: formulations

Sample preparation: Dissolve ointment in petroleum ether, add an equal volume of EtOH:water 70:30, dilute with MeOH to 100 μ g/mL, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m 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' 1.56

OTHER SUBSTANCES

Simultaneous: anhydrotetracycline, chlortetracycline, demeclocycline, doxycycline, epianhydrotetracycline, oxytetracycline, quatrimycin, rolitetracycline

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:** formulations**Sample preparation:** Dissolve a capsule in 1 L 100 mM HCl, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 7 μ m Zorbax TMS**Mobile phase:** DMF:water 10:90 containing 5 mM sodium ethylenediaminetetraacetate, 100 mM citric acid, 20 mM sodium citrate, and 50 mM potassium nitrate.**Flow rate:** 1.8**Injection volume:** 20**Detector:** UV 270

CHROMATOGRAM**Retention time:** 3.4

OTHER SUBSTANCES**Simultaneous:** sulfamethizole**Noninterfering:** phenazopyridine

KEY WORDS

capsules

REFERENCE

Du Preez, J.L.; Botha, S.A.; Lötter, A.P. High-performance liquid chromatographic determination of phenazopyridine hydrochloride, tetracycline hydrochloride and sulphamethizole in combination. *J.Chromatogr.*, **1985**, *333*, 249–252

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 \times 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 μ L EDTA buffer to the column, elute with 3 mL EDTA buffer, add 20 μ L 25 μ g/mL demeclocycline hydrochloride in MeOH to the eluate, inject a 100 μ L 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 Na₂HPO₄, 29.2 g NaCl, and 100 mmoles EDTA in 1 L water.)

HPLC VARIABLES**Guard column:** 5 \times 3 PLRP-S (Polymer Laboratories)**Column:** 250 \times 4.6 5 μ m 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 μ L reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water.)

CHROMATOGRAM**Retention time:** 5.9

Internal standard: demeclocycline (8.3)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline

KEY WORDS

protect from light; cow; post-column reaction; derivatization; SPE

REFERENCE

Croubels, S.; Van Peteghem, C.; Baeyens, W. Sensitive spectrofluorimetric determination of tetracycline residues in bovine milk. *Analyst*, **1994**, *119*, 2713–2716

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 μ L 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 μ L 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 Na₂HPO₄, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m 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: 13.3

Limit of detection: 0.52 ng/mL

Limit of quantitation: 1.10 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline, doxycycline, methacycline, minocycline, oxytetracycline

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. *JAOAC 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 μ m, PVDF), inject a 1000 μ L aliquot of the filtrate.

HPLC VARIABLES**Guard column:** 5 μ m PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)**Column:** 150 \times 4.6 5 μ m 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:** 18**Limit of detection:** 5 ng/mL

OTHER SUBSTANCES**Extracted:** chlortetracycline, 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:** Place 22 g 40 μ m, 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 μ L milk + 10 μ L 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 μ L 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 μ L mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μ m), inject a 20 μ L aliquot of the filtrate.

HPLC VARIABLES**Column:** 300 \times 4 10 μ m 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:** 4.5**Limit of detection:** 2 ng

OTHER SUBSTANCES

Extracted: chlortetracycline, 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: 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 Na₂HPO₄ 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: 10.8

Limit of detection: 11.6 ng/mL

Limit of quantitation: 31.3 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline

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: 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, dapson, 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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, 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, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phen-suximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyriethyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, 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 1 $\mu\text{g/mL}$ 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: 10

OTHER SUBSTANCES

Simultaneous: chlortetracycline, oxytetracycline

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

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: 8.5

OTHER SUBSTANCES

Simultaneous: chlortetracycline, oxytetracycline

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: 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 coil at 60° to the detector.

CHROMATOGRAM

Retention time: 13.7

Limit of detection: 20 ng/g (muscle); 230 ng/g (kidney)

OTHER SUBSTANCES

Extracted: chlortetracycline, oxytetracycline

KEY WORDS

pig; cow; poultry; kidney; muscle; SPE; post-column reaction

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

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 the 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 Na₂HPO₄·2H₂O + 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: 6.5

Limit of detection: 50 ng/g

OTHER SUBSTANCES

Extracted: chlortetracycline, 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: 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 (ethylenedinitrilotetraacetic 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: 6.5

Limit of quantitation: 10 ng/g

OTHER SUBSTANCES

Extracted: chlortetracycline, demethylchlortetracycline, doxycycline, methacycline, oxytetracycline

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

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Croubles, S.; Baeyens, W.; Van Peteghem, C. Evaluation of a narrow-bore HPLC column for trace level analysis of tetracyclines—a comparison with a conventional column. *Biomed.Chromatogr.*, **1995**, *9*, 251-253 [simultaneous chlortetracycline, demeclocycline, oxytetracycline]

Barker, S.A.; Long, A.R. Preparation of milk samples for immunoassay and liquid chromatographic screening using matrix solid-phase dispersion. *J.AOAC Int.*, **1994**, *77*, 848-854 [MSPD; infant formula; also albendazole, chloramphenicol, chlorsulon, chlortetracycline, fenbendazole, furazolidone, mebendazole, oxfendazole, oxytetracycline, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfa-

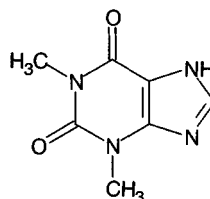
- methazine, sulfamethoxazole, sulfanilamide, sulfathiazole, sulfisoxazole, thiabendazole; LOD 0.1-2 ng]
- Muritu, J.W.; Kibwage, I.O.; Maitai, C.K.; Hoogmartens, J. Evaluation of tetracycline raw materials and finished products found on the Kenyan market. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1483–1488 [bulk; column temp 60; capsules; ointment; simultaneous impurities]
- Walsh, J.R.; Walker, L.V.; Webber, J.J. Determination of tetracyclines in bovine and porcine muscle by high-performance liquid chromatography using solid-phase extraction. *J.Chromatogr.*, **1992**, *596*, 211–216
- Ray, A.; Newton, V. Use of high-performance liquid chromatography to monitor stability of tetracycline and chlortetracycline in susceptibility determinations. *Antimicrob.Agents Chemother.*, **1991**, *35*, 1264–1266
- Khan, N.H.; Wera, P.; Roets, E.; Hoogmartens, J. Quantitative analysis of tetracycline by high performance liquid chromatography on polystyrene-divinylbenzene packing materials. *J.Liq.Chromatogr.*, **1990**, *13*, 1351–1374 [column temp 60; simultaneous impurities, acetyldecarboxamidotetracycline, anhydrotetracycline, epianhydrotetracycline, epitetracycline; bulk]
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- Vancaillie, R.; Dihuidi, K.; Roets, E.; Hoogmartens, J.; Vanderhaeghe, H. Determination by high performance liquid chromatography of the stability of alcoholic solutions for topical use containing tetracycline and 4-epitetracycline. *J.Pharm.Belg.*, **1985**, *40*, 168–172
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Theophylline

Molecular formula: C₇H₈N₄O₂

Molecular weight: 180.2

CAS Registry No.: 58-55-9, 32156-80-2 (diethanolamine), 573-41-1 (ethanolamine), 5600-19-1 (isopropanolamine), 8002-89-9 (sodium acetate), 8000-10-1 (sodium glycinate), 5967-84-0 (monohydrate)



SAMPLE

Matrix: bile, blood, tissue, urine

Sample preparation: Tissue. Macerate 2 g tissue with 5 mL water, add 15 mL MeCN, add 0.1 mL 1 mg/mL 2-acetamidophenol in ethanol, shake, centrifuge at 5200 g. Transfer supernatant to 50 mL tube containing 8 mL diethyl ether + 12 mL dichloromethane + 1 mL citrate buffer, vortex, proceed as in (A). Bile. 2 mL Bile + 3 mL water, add 15 mL MeCN, add 0.1 mL 1 mg/mL 2-acetamidophenol in ethanol, vortex, add 8 mL diethyl ether + 12 mL dichloromethane, vortex, centrifuge at 5200 g, proceed as in (A). Blood, urine. 5 mL Blood or urine + 15 mL acetone + 0.1 mL 1 mg/mL 2-acetamidophenol in ethanol, vortex for a few s, add 8 mL diethyl ether, vortex, centrifuge 5200 g. Transfer supernatant to 50 mL tube, add 12 mL dichloromethane, add 1 mL citrate buffer, vortex, proceed as in (A). (A). Discard lower, aqueous layer. Filter the organic layer through 3 g Florisil + 8 g anhydrous sodium sulfate and wash through with 15 mL diethyl ether. Evaporate filtrate to dryness under a stream of air at 40°. Reconstitute in 5 mL MeCN:0.1 N NaH₂PO₄ 60:40 + 3 mL hexane, vortex. Remove and discard upper hexane layer, add 8 mL 20% (v/v) isopropanol in chloroform to the aqueous layer, vortex. Remove and discard the upper aqueous layer and evaporate lower layer. Reconstitute residue in MeCN:water 10:90, inject an aliquot. (Citrate buffer was saturated sodium citrate containing enough sodium tustate (sic) to bring pH to 8. To each 1 L of diethyl ether 10 mL of water and 1 mL of citrate buffer are added.)

HPLC VARIABLES

Column: 100 × 4.6 C18 microbore

Mobile phase: MeCN:dilute phosphoric acid (1 mL 85% phosphoric acid in 140 mL water) 7:93

Column temperature: 40

Flow rate: 0.3

Detector: UV 271

CHROMATOGRAM

Retention time: 3.2

Internal standard: 2-acetamidophenol (4.9)

Limit of detection: 50 (blood, urine); 200 (bile, tissue) ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen

KEY WORDS

liver; whole blood

REFERENCE

Mathis, D.F.; Budd, R.D. Extraction of acetaminophen and theophylline from post-mortem tissues and urine for high-performance liquid chromatographic analysis. *J.Chromatogr.*, **1988**, *439*, 466–469

SAMPLE

Matrix: blood

Sample preparation: 100 μL Plasma + 10 μL 40 $\mu\text{g}/\text{mL}$ β -hydroxyethyltheophylline + 10 μL perchloric acid, vortex for 10 s, centrifuge for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 5 μm C18 Radpak (Waters)

Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 :triethylamine 5:7:88:0.02, pH 4.5

Flow rate: 3

Injection volume: 20

Detector: UV 273

CHROMATOGRAM

Retention time: 5.5

Internal standard: β -hydroxyethyltheophylline (7.5)

Limit of quantitation: 625 ng/mL

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Angus, P.W.; Ng, C.Y.; Ghabrial, H.; Morgan, D.J.; Smallwood, R.A. Effects of chronic left ventricular failure on hepatic oxygenation and theophylline elimination in the rat. *Drug Metab.Dispos.*, **1995**, *23*, 485–489

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 10 μL 2 mg/mL IS in MeOH, mix, add 2 mL dichloromethane:diethyl ether 80:20, vortex for 15 s, centrifuge at 1500 g for 5 min, remove a 1.7 mL aliquot of the organic phase, repeat the extraction twice more with 2 mL portions of dichloromethane:diethyl ether 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 \times 4.6 40 μm Pelliguard C18 (Supelco)

Column: 250 \times 4.6 5 μm Viosfer C18 (Violet, Rome)

Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 3 with orthophosphoric acid.)

Flow rate: 1.3

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 4.8

Internal standard: 2-[4-(2'-furoyl)phenyl]propionic acid (7.3)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: rufloxacin

KEY WORDS

plasma

REFERENCE

Carlucci, G.; Mazzeo, P.; Palumbo, G. Simultaneous determination of rufloxacin and theophylline by high-performance liquid chromatography in human plasma. *Analyst*, **1995**, *120*, 2493–2495

SAMPLE**Matrix:** blood**Sample preparation:** Add 200 μL whole blood to 400 μL MeCN while mixing rapidly for 5 s, centrifuge at 1500 rpm for 2 min, mix 100 μL supernatant with 1 mL water, inject a 50 μL aliquot.

HPLC VARIABLES**Column:** 50 \times 4.6 Little Champ reverse phase (Regis)**Mobile phase:** MeCN:10 mM pH 3 sodium phosphate buffer 4:96**Flow rate:** 1**Injection volume:** 50**Detector:** UV 273

CHROMATOGRAM**Limit of quantitation:** 500 nM

KEY WORDSrat; whole blood

REFERENCEHoffman, D.J.; Seifert, T.; Borre, A.; Nellans, H.N. Method to estimate the rate and extent of intestinal absorption in conscious rats using an absorption probe and portal blood sampling. *Pharm.Res.*, **1995**, *12*, 889–894

SAMPLE**Matrix:** blood**Sample preparation:** 100 μL Sample + 300 μL 7-(2-hydroxyethyl)theophylline in chloroform:isopropanol 50:50, extract. Remove 200 μL of the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** Bio-Sil ODS-5S**Mobile phase:** MeCN:THF:10 mM pH 4.75 sodium acetate 2:1:97**Detector:** UV 273

CHROMATOGRAM**Internal standard:** 7-(2-hydroxyethyl)theophylline**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Extracted:** paraxanthine**Noninterfering:** cephalosporins

REFERENCELeonard, H.; Campbell, J.; Malliaros, D.; Berg, M.; Houser, S.; McLaughlin, L. Comparison of a theophylline HPLC reference method with an automated chemiluminescent immunoassay (Abstract 122). *Ther Drug Monit.*, **1995**, *17*, 413

SAMPLE**Matrix:** blood**Sample preparation:** Extract 250 μL plasma 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

OTHER SUBSTANCES

Extracted: caffeine

KEY WORDS

plasma; pig; theophylline is IS

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: 100 µL Plasma + 50 µL 20 µg/mL β-hydroxyethyltheophylline in MeOH + 200 µL 67 mM pH 8 phosphate buffer + 5 mL chloroform, shake for 10 min, centrifuge at 850 g 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, inject a 20 µL aliquot.

HPLC VARIABLES

Column: reverse-phase

Mobile phase: MeCN:20 mM pH 5 acetate buffer 7:93

Flow rate: 1.5

Injection volume: 20

Detector: UV 275

CHROMATOGRAM

Internal standard: β-hydroxyethyltheophylline

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Nagai, N.; Furuhashi, M.; Ogata, H. Drug interactions between theophylline and H₂-antagonists, roxatidine acetate hydrochloride and cimetidine: Pharmacokinetic analysis in rats in vivo. *Biol.Pharm.Bull.*, **1995**, *18*, 1610–1613

SAMPLE

Matrix: blood

Sample preparation: 100 µL Plasma + 10 µL 40 µg/mL β-hydroxyethyltheophylline + 10 µL perchloric acid, vortex for 10 s, centrifuge for 5 min, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 5 µm Radpak C18 (Waters)

Mobile phase: MeCN:MeOH:buffer 5:7:88 (Buffer was 10 mM KH_2PO_4 containing 0.02% triethylamine, pH 4.5.)

Injection volume: 20

Detector: UV 273

CHROMATOGRAM

Retention time: 5.5

Internal standard: β -hydroxyethyltheophylline (7.5)

Limit of quantitation: 625 ng/mL

KEY WORDS

rat; plasma

REFERENCE

Ng, C.Y.; Angus, P.W.; Ghabrial, H.; Chou, S.T.; Arnolda, L.; Morgan, D.J.; Smallwood, R.A. Right heart failure impairs hepatic oxygenation and theophylline clearance in rats. *J.Pharm.Exp.Ther.*, **1995**, *273*, 1332–1336

SAMPLE

Matrix: blood

Sample preparation: Filter (0.22 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 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: 5.5

OTHER SUBSTANCES

Extracted: caffeine

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: 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: 9.4
Internal standard: β -hydroxyethyltheophylline (10.9)

OTHER SUBSTANCES

Extracted: caffeine, 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

Sample preparation: 200 μ L Serum + 10 μ L 500 ng/mL theophylline in water + 50 μ L isoamyl alcohol, vortex for 30 s, add 2 mL chloroform, vortex for 1 min, centrifuge at 1000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m Novapak C18

Mobile phase: MeCN:0.2% acetic acid 7.5:92.5 (After 4 min increase flow to 1.8 mL/min over 1 min, maintain at 1.8 mL/min for 2 min.)

Flow rate: 0.8

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 3.4

Internal standard: zidovudine (6.0)

Limit of detection: 20 ng/mL

KEY WORDS

rat; serum; pharmacokinetics

REFERENCE

Radwan, M.A. HPLC assay of theophylline and zidovudine in rat serum. *J.Liq.Chromatogr.*, **1995**, *18*, 3301-3309

SAMPLE

Matrix: blood

Sample preparation: Wash PCPure cartridge containing 0.4 g hydroxyapatite with 10 mL MeCN and remove MeCN by evaporation. 75 μ L Plasma + 25 μ L of 50 μ g/mL IS in 0.5% aqueous MeCN injected onto PCPure cartridge, elute with MeCN:water 10:90. Use first 600 μ L of eluate, inject 20 μ L aliquots.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Inertsil ODS-2

Mobile phase: MeCN:water 5:95
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7
Internal standard: 7-(2-hydroxyethyl)theophylline (10)
Limit of detection: 2.9 ng

OTHER SUBSTANCES

Simultaneous: caffeine

KEY WORDS

plasma

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 μ L Plasma + 100 μ L 20 μ g/mL caffeine + 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 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: caffeine

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: 100 μ L Plasma + 100 μ L pH 7.4 phosphate buffer + 50 μ L 20 μ g/mL β -hydroxypropyltheophylline in pH 7.4 phosphate buffer + 5 mL chloroform:isopropanol 95:5, shake on a rotary mixer for 15 min, centrifuge at 800 g for 5 min. Evaporate organic layer under nitrogen at 45°, sonicate residue with 100 μ L mobile phase, inject 25 μ L aliquot.

HPLC VARIABLES**Guard column:** 10 × 4.9 Spherisorb ODS**Column:** 250 × 4.9 Spherisorb S5 ODS2**Mobile phase:** MeCN:buffer 15:85 adjusted to pH 3.0 with 85% phosphoric acid immediately before use (Buffer was 4.54 g KH₂PO₄ + 5.94 g Na₂HPO₄·2H₂O + 1.49 g tetrabutylammonium hydrogen sulfate per L.)**Flow rate:** 1.3**Injection volume:** 25**Detector:** UV 280

CHROMATOGRAM**Retention time:** 3.3**Internal standard:** β-hydroxypropyltheophylline**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Extracted:** ciprofloxacin, enoxacin, norfloxacin

KEY WORDS

plasma; rat

REFERENCEDavis, J.D.; Aarons, L.; Houston, J.B. Simultaneous assay of fluoroquinolones and theophylline in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1993**, *621*, 105–109

SAMPLE**Matrix:** blood**Sample preparation:** 200 μL Serum + 200 μL 20 μg/mL antipyrine in 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 C₁₂ DAPS (Fluka) 3:97 (C₁₂ DAPS is 3-(dimethyldodecylammonio) propanesulfonate.)**Injection volume:** 25**Detector:** UV 273

CHROMATOGRAM**Retention time:** 5**Internal standard:** antipyrine (8)**Limit of detection:** 500 ng/mL

OTHER SUBSTANCES**Simultaneous:** albendazole, albendazole sulfoxide, aminophylline, amyleine, caffeine, flubendazole, β-hydroxytheophylline, mercaptopurine, nimorazole, procaine, theobromine**Interfering:** dipropylamine, metronidazole, tinidazole

KEY WORDS

serum; micellar chromatography

REFERENCEHabel, 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:** 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:** 2.4**Internal standard:** theobromine (3.3)

OTHER SUBSTANCES**Simultaneous:** caffeine

KEY WORDS

serum

REFERENCEHelmsing, P.J.; Huisman, R.; van der Weele, A. HPLC determination of caffeine and theophylline by direct serum injection. *Clin.Chem.*, **1993**, *39*, 1348–1349

SAMPLE**Matrix:** blood**Sample preparation:** 10 µL Plasma + 300 µL 100 mM pH 6.0 KH₂PO₄ 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 KH₂PO₄ 9:2.5:90**Flow rate:** 2**Injection volume:** 25**Detector:** E, ESA Coulochem Model 5100 A, Model 5010 analytical cell, first (screen) electrode 0.68 V, second (measuring) electrode 0.98 V, Model 5020 guard cell (before injector) 1.0 V; UV 270

CHROMATOGRAM**Retention time:** 8 (electrochemical detection)**Internal standard:** diprophylline (UV detection) (10)**Limit of detection:** 200 ng/mL

OTHER SUBSTANCES**Extracted:** caffeine, 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 μ L Serum + 100 μ L 20 μ g/mL hydroxyethyltheophylline in 2 M perchloric acid, vortex, centrifuge 5 min, inject 50 μ L aliquot of supernatant.

HPLC VARIABLES

Column: 125 \times 4 LiChroSpher RP-8 5 μ m

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: 4.6

Internal standard: hydroxyethyltheophylline (5.6)

OTHER SUBSTANCES

Simultaneous: caffeine, 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 μ L Plasma + 50 μ L 3 μ g/mL 8-chlorotheophylline in mobile phase + 100 μ L 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 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m TSK gel ODS-80TM (Tosoh)

Mobile phase: MeOH:100 mM NaH₂PO₄ 30:70

Flow rate: 0.8

Injection volume: 20

Detector: UV 274

CHROMATOGRAM

Retention time: 5.9

Internal standard: 8-chlorotheophylline (11.7)

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, caffeine, paraxanthine, theobromine

Noninterfering: acetaminophen, aspirin, chlorpheniramine, etenzamide, hydrocortisone, phenacetin, phenobarbital, phenylbutazone, phenytoin, prednisolone, prednisone, salicylic acid, trimethadione, vitamin B1

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: 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 \times 4.6 IRSP silica (for preparation see *Anal. Chem.* 1989, 61, 2445); B 150 \times 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: 7.5

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, theobromine

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: 100 μL Plasma or serum + 35 μL 22 $\mu\text{g}/\text{mL}$ 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 \times 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:** 15.60**Internal standard:** 3-ethylxanthine (13.64)

OTHER SUBSTANCES**Extracted:** acetaminophen, caffeine, dimethyluric acids, methylxanthines, paraxanthine, theobromine, trimethyluric acid

KEY WORDS

plasma; serum; SPE

REFERENCELeakey, 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:** Centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μm internal-surface, reversed-phase, Pinkerton-type, 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:** 10.88**Limit of detection:** <1000 ng/mL

OTHER SUBSTANCES**Extracted:** caffeine, doxofylline, dyphylline**Noninterfering:** 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, mepidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, nicotine, nordiazepam, nortriptyline, orphenadrine, papaverine, pentazocine, phenacetin, phenacyclidine, 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, tripeleminamine**Interfering:** acetaminophen

KEY WORDS

serum; plasma; direct injection

REFERENCETagliaro, 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:** 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 μ L plasma then 50 μ L 10 μ g/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 μ L mobile phase, inject a 15 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Supelcosil-LC-8**Mobile phase:** MeCN:water 20:80**Flow rate:** 3.3**Injection volume:** 15**Detector:** UV 208

CHROMATOGRAM**Retention time:** 0.77**Internal standard:** tolylphenobarbital (7.57)**Limit of detection:** 50-100 ng/mL

OTHER SUBSTANCES**Extracted:** amobarbital, barbital, butabarbital, caffeine, carbamazepine epoxide, carbamazepine, carbamazepinediol, chloramphenicol, ethosuximide, glutethimide, mephentoin, methaqualone, methyprylon, nirvanol, pentobarbital, phenacemide, phenobarbital, phenytoin, primidone, secobarbital**Noninterfering:** acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, p-hydroxyphenobarbital, imipramine, lidocaine, methotrexate, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, vancomycin

KEY WORDS

plasma; SPE

REFERENCESvinarov, 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 μ L Serum + 100 μ L 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 μ L mobile phase, inject a 6-10 μ L 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 \times 4.6 Supelguard LC-1 (Supelco)**Column:** 250 \times 4.6 5 μ m 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:** 1.95**Internal standard:** 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES**Extracted:** acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, 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, mephénytoin, 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

REFERENCEMeatherall, 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:** 200 μ L Serum + 400 μ L 100 μ g/mL 8-chlorotheophylline in MeCN, vortex for 10 s, centrifuge at 15000 rpm for 3 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Ultrasphere ODS**Mobile phase:** MeCN:water:glacial acetic acid 4:84:12 containing 4.84 g/L Trizma, pH 2.3**Flow rate:** 2**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 3.00**Internal standard:** 8-chlorotheophylline (5.29)**Limit of quantitation:** 2 μ g/mL

OTHER SUBSTANCES**Extracted:** acetaminophen, salicylic acid**Simultaneous:** caffeine, cefazolin, cimetidine, ergotamine, glutethimide, heparin, methamphetamine, propranolol, sulfamethoxazole, theobromine, tobutamide, trimethoprim**Noninterfering:** amitriptyline, amobarbital, ampicillin, butabarbital, butalbital, celbenine, chlordiazepoxide, chlorpromazine, clorazepate, desipramine, diazepam, doxepin, ethchlorvynol, fluphenazine, hydroxyzine, ibuprofen, imipramine, isoniazid, lidocaine, mephobarbital, mesoridazine, methaqualone, methyluric acid, naprotyline, nordiazepam, nortriptyline, oxazepam, pentobarbital, perphenazine, phenelzine, phenmetrazine, phenobarbital, phenylbutazone, phenytoin, prednisolone, prednisone, procainamide, prochlorperazine, promazine, promethazine, propoxyphene, protriptyline, pyrilamine, secobarbital, thioridazine, thiothixene, timolol, trazodone, triazolam, trifluoperazine

KEY WORDS

serum

REFERENCE

Osterloh, J.; Yu, S. Simultaneous ion-pair and partition liquid chromatography of acetaminophen, theophylline and salicylate with application to 500 toxicologic specimens. *Clin.Chim.Acta*, **1988**, *175*, 239-248

SAMPLE**Matrix:** blood

Sample preparation: 100 μ L 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 μ L mobile phase, inject a 40 μ L aliquot. (Reagent was 1.5 mg β -hydroxyethyltheophylline and 25 μ L glacial acetic acid in 100 mL chloroform:isopropanol 95:5.)

HPLC VARIABLES**Guard column:** 30 mm long 5 μ m Ultrasphere ion-pair**Column:** 150 \times 4.6 5 μ m 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 \pm 0.03 with concentrated HCl

Flow rate: 1.2**Injection volume:** 40**Detector:** UV 280

CHROMATOGRAM**Retention time:** k' 5.4**Internal standard:** β -hydroxyethyltheophylline (k' 4.2)

OTHER SUBSTANCES**Extracted:** caffeine, 1,7-dimethyl xanthine, theobromine

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: 200 μ L Serum + 200 μ L 4 μ g/mL 8-chlorotheophylline in MeCN, mix, centrifuge, evaporate the supernatant to dryness, reconstitute in 400 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 100 \times 3 8 μ m 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: 3.8
Internal standard: 8-chlorotheophylline (8.8)
Limit of detection: 1 µg/mL

OTHER SUBSTANCES

Simultaneous: caffeine

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: 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: 5.95
Internal standard: 8-chlorotheophylline (8.85)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, dyphylline, paraxanthine, proxyphylline
Simultaneous: cefoxitin
Noninterfering: carbenicillin, cefoperazone, cephacetril, heparin, penicillin G, phenytoin, phenobarbital

KEY WORDS

serum; plasma; 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:** 50 μL Serum + 50 μL 15 $\mu\text{g}/\text{mL}$ 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:** μ Bondapak 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, N-acetylprocainamide, aspirin, procainamide, salicylic acid**Noninterfering:** benzoic acid**Interfering:** dyphylline (separated with MeCN:buffer 8:92), ampicillin

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 + β -hydroxypropyltheophylline, 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 \times 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:** 4.5**Internal standard:** β -hydroxypropyltheophylline (3.7)

OTHER SUBSTANCES**Simultaneous:** caffeine, dyphylline, theobromine**Noninterfering:** acetaminophen, acetazolamide, albuterol, amitriptyline, amobarbital, carbamazepine, diazoxide, disopyramide, ethosuximide, isoproterenol, nitrazepam, nortriptyline, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, salicylic acid, sulthiame

KEY WORDS

serum

REFERENCEPaterson, 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:** 200 μ L Serum + 200 μ L 50 μ g/mL hexobarbital in MeCN + 25 μ L glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** μ Bondapak 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:** 4.3**Internal standard:** hexobarbital (20.6)**Limit of detection:** LOD 200-2000 ng/mL

OTHER SUBSTANCES**Extracted:** acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital**Simultaneous:** amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCEKabra, 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: 1 mL Plasma + 100 μ L 0.2 mg/mL β -hydroxyethyltheophylline in buffer + 100 μ L 40% aqueous trichloroacetic acid, vortex for 30 s, let stand for 5 min, centrifuge at 2000 g for 15 min, inject a 25 μ L aliquot of the supernatant. (Buffer was 10 mM sodium acetate adjusted to pH 4.0 with glacial acetic acid.)

HPLC VARIABLES

Column: 10 μ m μ Bondapak 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: 6.5

Internal standard: β -hydroxyethyltheophylline (9)

Limit of detection: 1 μ g/mL

OTHER SUBSTANCES

Extracted: caffeine, dyphylline, 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, CSF, gastric fluid, urine

Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m 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

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiaze-poxide, 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, 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 juice, pancreatic juice

Sample preparation: 500 μ L Serum, pancreatic juice, or gastric juice + 25 μ L 200 μ g/mL β -hydroxyethyltheophylline in water + 500 μ L 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: C18 LichroCART

Column: 250 \times 4.6 7 μ m Lichrosorb C18

Mobile phase: THF:MeOH:10 mM pH 3.5 KH₂PO₄ 1:20:79

Flow rate: 0.8

Injection volume: 50

Detector: UV 280

CHROMATOGRAM

Retention time: 7.03

Internal standard: β -hydroxyethyltheophylline (7.48)

Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, 1-methyluric acid, 1-methylxanthine, paraxanthine, theobromine

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, saliva

Sample preparation: 500 μ L Plasma or saliva + 50 μ L 50 ng/mL difloxacin, vortex briefly, add 500 μ L 100 mM pH 7.4 phosphate buffer, add 4 mL dichloromethane, add 1 mL isopropanol, vortex for 30 s, shake gently for 30 min, centrifuge at 1500 g for 20 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 μ L mobile phase, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 Guard-Pak

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:buffer 35:100 (Buffer was 5.44 g KH_2PO_4 and 4 mL tetrabutylammonium hydroxide in 1 L water, adjust pH to 2.5 with 85% phosphoric acid.)

Flow rate: 2

Injection volume: 50-200

Detector: UV 268

CHROMATOGRAM

Retention time: 4.3

Internal standard: difloxacin (8.8)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: ciprofloxacin, enoxacin

Simultaneous: caffeine

Noninterfering: 1,3-dimethyluric acid, hypoxanthine, 1-methyluric acid, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, theobromine

Interfering: 1,7-dimethylxanthine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Zhai, S.; Korrapati, M.R.; Wei, X.; Muppalla, S.; Vestal, R.E. Simultaneous determination of theophylline, enoxacin and ciprofloxacin in human plasma and saliva by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 669, 372-376

SAMPLE

Matrix: blood, saliva

Sample preparation: 100 μL Serum, plasma, or saliva + 100 μL 15 $\mu\text{g}/\text{mL}$ 8-chlorotheophylline in 6% perchloric acid, vortex for 10 s, centrifuge at 10800 g for 10 min, inject 20 μL of the clear supernatant.

HPLC VARIABLES

Guard column: Whatman Co:Pell ODS

Column: 300 \times 3.9 10 μm Bondex ODS (Phenomenex)

Mobile phase: THF:buffer 6:94 (Buffer was pH 4.0 10 mM, prepared from 41 mL 200 mM acetic acid and 9 mL 200 mM sodium acetate made up to 1 L with water, pH adjusted to 4.0 with glacial acetic acid or 100 mM NaOH if necessary.)

Flow rate: 1

Injection volume: 20

Detector: UV 273

CHROMATOGRAM

Retention time: 4.7

Internal standard: 8-chlorotheophylline (11)

Limit of quantitation: 70 ng/mL

OTHER SUBSTANCES

Simultaneous: caffeine, paraxanthine, theobromine

KEY WORDS

serum; plasma

REFERENCE

Blanchard, J.; Harvey, S.; Morgan, W.J. A rapid and specific high-performance liquid chromatographic assay for theophylline in biological fluids. *J.Chromatogr.Sci.*, **1990**, 28, 303-306

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 μ L Supernatant + 100 μ L 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 μ L mobile phase, vortex for 1 min, inject a 25 μ L aliquot. Serum. 200 μ L Serum + 100 μ L 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 μ L mobile phase, vortex for 1 min, inject a 25 μ L aliquot.

HPLC VARIABLES

Guard column: 10 μ m μ Bondapak C18

Column: 250 \times 4.6 10 μ m μ Bondapak C18

Mobile phase: THF:10 mM Na₂HPO₄ 3:97, pH adjusted to 6.5 with phosphoric acid

Flow rate: 2.5

Injection volume: 25

Detector: UV 273

CHROMATOGRAM

Retention time: 5

Limit of detection: 62.5 ng/g (tissue); 62.5 ng/mL (serum)

OTHER SUBSTANCES

Extracted: caffeine, paraxanthine, theobromine

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: Plasma. 500 μ L Plasma + 25 μ L 300 μ M IS in EtOH:water 30:70 + 75 μ L 1 M HCl, mix, add 5 mL ethyl acetate:isopropanol 90:10, shake for 10 min, centrifuge at 1000 g for 10 min, freeze at -30°. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 300 μ L mobile phase, sonicate until dissolved, centrifuge at 13000 g for 15 min, inject a 40 μ L aliquot. Urine. Acidify 10 mL urine with 300 μ L 1 M HCl. 40 μ L Acidified urine + 50 μ L 300 μ M IS in EtOH:water 30:70, mix, make up to 400 μ L with 10 mM pH 4.0 acetate buffer, add 5 mL ethyl acetate:isopropanol 93:7, shake for 10 min, centrifuge at 1000 g for 10 min, freeze at -30°. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 300 μ L mobile phase, vortex for 10 s, inject a 40 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeOH:10 mM pH 4.0 acetate buffer 9:91 (plasma) or 7:93 (urine)

Column temperature: 30

Flow rate: 1 for 11 min, 1.5 for 6 min, 2.5 for 13 min

Injection volume: 40

Detector: UV 273

CHROMATOGRAM

Retention time: 20.2 (plasma), 20.9 (urine)

Internal standard: β -hydroxyethyltheophylline (24.2 (plasma), 28.4 (urine))

Limit of detection: 200 nM (plasma); 2 μ M (urine)

OTHER SUBSTANCES

Extracted: metabolites, 1,3-dimethyluric acid, 1-methyluric acid, 3-methylxanthine

KEY WORDS

plasma

REFERENCE

Rasmussen, B.B.; Brosen, K. Determination of theophylline and its metabolites in human urine and plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1996**, 676, 169–174

SAMPLE

Matrix: formulations

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 10 μ m Dynamax C18

Column: 250 \times 4.6 10 μ m Dynamax C18

Mobile phase: MeCN:6.5 mM tetraheptylammonium bromide:100 mM pH 7.0 phosphate buffer:100 mM pH 5.0 citrate buffer 40:55.2:4.4:0.4

Flow rate: 1

Injection volume: 20

Detector: UV 271

CHROMATOGRAM

Retention time: 3.0

OTHER SUBSTANCES

Simultaneous: degradation products, ceftriaxone

KEY WORDS

injections; use low actinic glassware; stability-indicating; saline; 5% dextrose

REFERENCE

Parrish, M.A.; Bailey, L.C.; Medwick, T. Stability of ceftriaxone sodium and aminophylline or theophylline in intravenous mixtures. *Am.J.Hosp.Pharm.*, **1994**, 51, 92–94

SAMPLE

Matrix: formulations

Sample preparation: Dilute to 0.8-13 μ g/mL, inject an aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrasphere C18

Mobile phase: MeCN:100 mM pH 3.4 acetate buffer 1:10 (Buffer was 50 mL 100 mM sodium acetate diluted to 1 L with 100 mM acetic acid.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Internal standard: 5-methylresorcinol

OTHER SUBSTANCES

Simultaneous: aminophylline, cefuroxime

KEY WORDS

injections; 5% dextrose

REFERENCE

Stewart, J.T.; Warren, F.W.; Johnson, S.M. Stability of cefuroxime sodium and aminophylline or theophylline. *Am.J.Hosp.Pharm.*, **1994**, *51*, 809–811

SAMPLE

Matrix: formulations

Sample preparation: Shake, remove 2 mL of oral suspension, dilute to 40 mL with water, vortex 1 min, centrifuge at 2000 rpm for 10 min. Dilute a 100 μ L aliquot of supernatant with 100 μ L of 100 μ g/mL theophylline and add 800 μ L water, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 300 mm 10 μ m Waters reversed-phase C18

Mobile phase: MeCN:50 mM sodium acetate buffer 8:92, adjusted to pH 6.5

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4.3

Internal standard: theophylline

OTHER SUBSTANCES

Simultaneous: famotidine

KEY WORDS

stability-indicating; oral suspensions; theophylline is IS

REFERENCE

Quercia, R.A.; Jay, G.T.; Fan, C.; Chow, M.S. Stability of famotidine in an extemporaneously prepared oral liquid. *Am.J.Hosp.Pharm.*, **1993**, *50*, 691–693

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 \times 2.1 Co:Pell ODS

Column: 300 \times 3.9 10 μ m μ Bondapak 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 (mobile phase B), 7.45 (mobile phase A)

OTHER SUBSTANCES

Simultaneous: ethylenediamine

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: microsomal incubations

Sample preparation: 500 μ L Microsomal incubation + 400 μ L 2% zinc sulfate, centrifuge at 5000 rpm for 5 min. Remove the supernatant and add it to 30 mg ammonium sulfate and 10 μ L 10 μ g/mL 1,7-dimethyluric acid, extract with dichloromethane:isopropanol 80:20. Remove the organic layer and evaporate it to dryness, reconstitute, inject an aliquot.

HPLC VARIABLES

Guard column: C18

Column: 250 \times 4.6 Spherex 5 C18 (Phenomenex)

Mobile phase: Gradient. MeCN:25 μ M pH 4.5 acetate buffer 2:98 for 20 min, 5:95 for 12 min, 9:91 for 8 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 1

Detector: UV 276

CHROMATOGRAM

Retention time: 35.5

Internal standard: 1,7-dimethyluric acid (33.6)

OTHER SUBSTANCES

Extracted: metabolites, 1,3-dimethyluric acid, 1-methylxanthine, 3-methylxanthine

KEY WORDS

human; liver

REFERENCE

Tjia, J.F.; Colbert, J.; Back, D.J. Theophylline metabolism in human liver microsomes: Inhibition studies. *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 912-917

SAMPLE

Matrix: microsomal incubations

Sample preparation: 275 μ L Microsomal incubation + 50 μ L 30% perchloric acid, centrifuge at 2000 g for 10 min, inject a 60 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 \times 4.6 40 μ m 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: 14

OTHER SUBSTANCES

Extracted: caffeine, paraxanthine, theobromine, 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 proxiphylline 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: 22.5

Internal standard: proxiphylline (25)

OTHER SUBSTANCES

Simultaneous: caffeine, paraxanthine, theobromine

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: perfusate

Sample preparation: Inject a 100 μL aliquot directly.

HPLC VARIABLES

Column: 250 × 4.6 octyl Spherisorb S5-ODS

Mobile phase: MeCN:100 mM KH₂PO₄ 10:90
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 272

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: saliva

Sample preparation: Centrifuge saliva at 10000 rpm for 2 min. 1 mL Supernatant + 50 μ L 80 μ g/mL β -hydroxyethyltheophylline + 100 μ L 100 mM HCl + 7 mL chloroform: isopropanol 95:5, shake for 10 min, centrifuge at 3000 rpm for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μ L mobile phase, filter, inject a 10 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 150 \times 4.6 ODS 120T (Tosoh)
Mobile phase: MeCN:10 mM pH 4.0 acetate buffer 9:91
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM

Internal standard: β -hydroxyethyltheophylline

KEY WORDS

pharmacokinetics

REFERENCE

Kanke, M.; Katayama, H.; Nakamura, M. Application of curdlan to controlled drug delivery. II. In vitro and in vivo drug release studies of theophylline-containing curdlan tablets. *Biol.Pharm.Bull.*, **1995**, *18*, 1104-1108

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 6.5 μ m 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: 13.5

Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES

Simultaneous: caffeine, hypoxanthine, pentoxifylline, propentofylline, theobromine, uric acid, xanthine

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: 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: 4.52 (A), 3.45 (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, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydroalazine, 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, methylodopa, methylphenidate, methoclopramide, 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, pimozone, 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, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinamide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 μ L aliquot of a solution in pH 7.4 Tyrode's buffer.

HPLC VARIABLES

Column: 125 \times 4.5 μ m 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: cefazolin

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

HPLC VARIABLES

Column: 250 \times 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, bromazepam, 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, diphenoxy-

late, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, 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, isocarboxystiril, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phen-suximide, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, 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 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: 7

OTHER SUBSTANCES

Simultaneous: caffeine, 8-chlorotheophylline, diphylline, theobromine

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 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: 1.04

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotinine, 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, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phenoltamine, 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, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazo-

done, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, 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, inject a 1 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 1.3 μ m 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: 3.56

OTHER SUBSTANCES

Also analyzed: acetaminophen, aspirin, buccetin (3-hydroxy-p-butyrophenetidine), caffeine, dipyrone (sulpyrin), ethenzamide (o-ethoxybenzamide), mefenamic acid, phenacetin, salicylamide, salicylic acid, theobromine

KEY WORDS

semi-micro; porous polymer

REFERENCE

Matsushima, Y.; Nagata, Y.; Niyomura, M.; Takakusagi, K.; Takai, N. Analysis of antipyretics by semi-micro liquid chromatography. *J.Chromatogr.*, **1985**, *332*, 269–273

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 10-fold with 5 μ g/mL β -hydroxyethyltheophylline in water, mix, centrifuge at 14000 rpm for 2 min, inject a 25 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 40 \times 2.5 μ m Lichrosorb RP-2

Column: 150 \times 4.6 μ m 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: 9

Internal standard: β -hydroxyethyltheophylline (11)

Limit of detection: 1 μ g/mL

OTHER SUBSTANCES

Extracted: metabolites, caffeine, 1,3-dimethyluric acid, 1-methyluric acid, 3-methylxanthine

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

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Hieda, Y.; Kashimura, S.; Hara, K.; Kageura, M. Highly sensitive and rapid determination of theophylline, theobromine and caffeine in human plasma and urine by gradient capillary high-performance liquid chromatography-frit-fast atom bombardment mass spectrometry. *J.Chromatogr.B*, **1995**, *667*, 241–246 [LC-MS; LOD 5 ng/mL]

Theodoridis, G.; Papadoyannis, I.; Vasilikiotis, G.; Tsoukali-Papadopoulou, H. Reversed-phase high-performance liquid chromatography–photodiode-array analysis of alkaloid drugs of forensic interest. *J.Chromatogr.B*, **1995**, *668*, 253–263 [also amphetamine, bamifylline, caffeine, cocaine, codeine, diamorphine, ethylmorphine, flufenamic acid, hyoscyamine, methadone, morphine, nalorphine, norcodeine, papaverine, quinine, scopolamine, strychnine, theobromine, tolfenamic acid]

Van den Mooter, G.; Samyn, C.; Kinget, R. *In vivo* evaluation of a colon-specific drug delivery system: An absorption study of theophylline from capsules coated with azo polymers in rats. *Pharm.Res.*, **1995**, *12*, 244–247 [plasma; LOD 250 ng/mL]

Hieda, Y.; Kashimura, S.; Hara, K.; Kageura, M. [Development of the method for analysis of theophylline and its related compounds using capillary high-performance liquid chromatography/fast atom bombardment-mass spectrometry]. *Nippon Hoigaku Zasshi*, **1994**, *48*, 253–262

Kanda, T.; Kutsuna, H.; Ohtsu, Y.; Yamaguchi, M. Synthesis of polymer-coated mixed-functional packing materials for direct analysis of drug-containing serum and plasma by high-performance liquid chromatography. *J.Chromatogr.A*, **1994**, *672*, 51–57 [direct injection; column temp 40; also carbamazepine, chloramphenicol, indomethacin, phenobarbital, phenytoin, trimethoprim]

Sarkar, M.A.; Jackson, B.J. Theophylline N-demethylations as probes for P4501A1 and P4501A2. *Drug Metab.Dispos.*, **1994**, *22*, 827–834 [theobromine (IS); LOD 50 ng/mL]

Smigol, V.; Svec, F.; Fréchet, J.M.J. Novel uniformly sized polymeric stationary phase with hydrophilized large pores for direct injection HPLC determination of drugs in biological fluids. *J.Liq.Chromatogr.*, **1994**, *17*, 891–911 [also aspirin, caffeine, carbamazepine, lidocaine, phenytoin, salicylic acid, theobromine]

Zhang, H.; Stewart, J.T. Determination of a cefuroxime and aminophylline/theophylline mixture by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1994**, *17*, 1327–1335 [simultaneous cefuroxime, orcinol]

Chow, A.T.; Meek, P.D.; Jusko, W.J. High-pressure liquid chromatographic assay of theophylline in dog feces following oral administration of sustained-release products. *J.Pharm.Sci.*, **1993**, *82*, 956–958

Abdel-Hay, M.H.; el-Din, M.S.; Abuirjeie, M.A. Simultaneous determination of theophylline and guaifenesin by third-derivative ultraviolet spectrophotometry and high-performance liquid chromatography. *Analyst*, **1992**, *117*, 157–160

Abuirjeie, M.A.; el-Din, M.S.; Mahmoud, I.I. Determination of theobromine, theophylline and caffeine in various food products using derivative UV-spectrophotometric techniques and high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1992**, *15*, 101–125 [ethyl paraben (IS)]

Mazzei, M.; Sottofattori, E.; Balbi, A.; Bottino, G.B. HPLC analysis of theophylline: bioequivalence study of two sustained-release formulations at steady state. *Farmaco*, **1992**, *47*, 769–777

Papadoyannis, I.; Georgarakis, M.; Samanidou, V.; Theodoridis, G. High-performance liquid chromatographic analysis of theophylline in the presence of caffeine in blood serum and pharmaceutical formulations. *J.Liq.Chromatogr.*, **1991**, *14*, 1587–1603

El-Sayed, Y.M.; Islam, S.I. High-performance liquid chromatographic analysis of theophylline in serum and its use in therapeutic drug monitoring. *J.Clin.Pharm.Ther.*, **1989**, *14*, 35–43

- Lam, S.; Malikin, G. An improved micro-scale protein precipitation procedure for HPLC assay of therapeutic drugs in serum. *J.Liq.Chromatogr.*, **1989**, *12*, 1851–1872 [also acetaminophen, amiodarone, aspirin, caffeine, chloramphenicol, flecainide, pentobarbital, procainamide, pyrimethamine, quinidine, tocainide, trazodone; fluorescence detection; UV detection]
- Thomas, J. [Analysis of theophylline and its sodium (or potassium) anisates by ultrafast high-performance liquid chromatography in different pharmaceutical preparations]. *J.Chromatogr.*, **1989**, *479*, 430–436
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- Kester, M.B.; Saccar, C.L.; Mansmann, H.C., Jr. A new simplified microassay for the quantitation of theophylline in serum by high-performance liquid chromatography. *J.Liq.Chromatogr.*, **1987**, *10*, 957–975 [LOD 100 ng/mL; extracted metabolites, caffeine, dimethyluric acid, dimethylxanthine, methylxanthine]
- Kester, M.B.; Saccar, C.L.; Mansmann, H.C., Jr. Microassay for the simultaneous determination of theophylline and dyphylline in serum by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *416*, 91–97
- Kester, M.B.; Saccar, C.L.; Rocci, M.L.J.; Mansmann, H.C., Jr. A new simplified assay for the quantitation of theophylline and its major metabolites in urine by high-performance liquid chromatography. *J.Pharm.Sci.*, **1987**, *76*, 238–241
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- Alvi, S.U.; Castro, F. A simultaneous assay of theophylline, ephedrine hydrochloride, and phenobarbital in suspensions and tablets formulations by high performance liquid chromatography. *J.Liq.Chromatogr.*, **1986**, *9*, 2269–2279 [guaifenesin (IS)]
- Wong, S.H.Y.; Marzouk, N.; McHugh, S.L.; Cazes, E. Simultaneous determination of theophylline and caffeine by reversed phase liquid chromatography using phenyl column. *J.Liq.Chromatogr.*, **1985**, *8*, 1797–1816 [hydroxyethyltheophylline (IS); also acetaminophen, cimetidine, codeine, dimethylxanthine, meperidine, pentobarbital, phenobarbital, secobarbital]
- Ong, H.; Marleau, S. A bidimensional HPLC system for direct determination of theophylline in serum. *J.Liq.Chromatogr.*, **1984**, *7*, 779–791 [column-switching]
- St-Pierre, M.V.; Tesoro, A.; Spino, M.; MacLeod, S.M. An HPLC method for the determination of theophylline and its metabolites in serum and urine. *J.Liq.Chromatogr.*, **1984**, *7*, 1593–1608 [extracted dimethyluric acid, methyluric acid, methylxanthine]
- Klassen, R.; Stavric, B. HPLC separation of theophylline, paraxanthine, theobromine, caffeine and other caffeine metabolites in biological fluids. *J.Liq.Chromatogr.*, **1983**, *6*, 895–906 [plasma; milk; urine; saliva]
- Ou, C.N.; Frawley, V.L. Concurrent measurement of theophylline and caffeine in neonates by an interference-free liquid-chromatographic method. *Clin.Chem.*, **1983**, *29*, 1934–1936
- Agostini, O.; Chiari, A.; Ciofi Baffoni, D. Simultaneous high-performance liquid chromatographic determination of salbutamol sulphate, theophylline, and saccharin in a hydroalcoholic formulation. *Boll.Chim.Farm.*, **1982**, *121*, 612–618
- Chen, T.M.; Chafetz, L. High-pressure liquid chromatographic assay of theophylline, ephedrine hydrochloride, and phenobarbital tablets. *J.Pharm.Sci.*, **1981**, *70*, 804–806
- Lurie, I.S.; Demchuk, S.M. Optimization of a reverse phase ion-pair chromatographic separation for drugs of forensic interest. II. Factors effecting selectivity. *J.Liq.Chromatogr.*, **1981**, *4*, 357–374 [also acetaminophen, acetylcodeine, acetylmorphine, aminopyrene, aminopyrine, amobarbital, amphetamine, antipyrine, benzocaine, butabarbital, caffeine, cocaine, codeine, diamorphine, diazepam, diethylpropion, DMT, ephedrine, glutethimide, Lampa, lidocaine, LSD, MDA, mecloqualone, mescaline, methamphetamine, methapyrilene, methaqualone, methylpyrilene, methylphenidate, morphine, narcotine, papaverine, PCP, pentobarbital, phencyclidine, phendimetrazine, phenmetrazine, phenobarbital, phentermine, phenylpropanolamine, procaine, quinidine, quinine, secobarbital, strychnine, TCP, tetracaine, thebaine]
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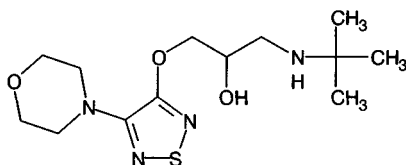
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- Tan, H.S.; Booncong, P.C.; Fine, S.L. Simultaneous high-performance liquid chromatographic determination of theophylline, ephedrine hydrochloride, and phenobarbital in tablets. *J.Pharm.Sci.*, **1981**, *70*, 783–785
- Van Aerde, P.; Moerman, E.; Van Severen, R.; Braeckman, P. Determination of plasma theophylline by straight-phase high-performance liquid chromatography: elimination of interfering caffeine metabolites. *J.Chromatogr.*, **1981**, *222*, 467–474
- Weidner, N.; Dietzler, D.N.; Ladenson, J.H.; Kessler, G.; Larson, L.; Smith, C.H.; James, T.; McDonald, J.M. A clinically applicable high-pressure liquid chromatographic method for measurement of serum theophylline, with detailed evaluation of interferences. *Am.J.Clin.Pathol.*, **1980**, *73*, 79–86

Timolol

Molecular formula: C₁₃H₂₄N₄O₃S

Molecular weight: 316.4

CAS Registry No.: 26839-75-8, 91524-16-2 (hemihydrate),
26921-17-5 (maleate)



SAMPLE

Matrix: aqueous humor

Sample preparation: 150 μ L Aqueous humor + 50 μ L 5 μ g/mL aniline in water + 100 μ L 400 mM NaOH, swirl-mix, add 3 mL isopropanol, swirl-mix for 2 min, centrifuge at 300 g for 5 min. Remove the organic layer and add it to 50 μ L 100 mM HCl, swirl-mix for 2 min, centrifuge, inject a 20 μ L aliquot of the acid layer.

HPLC VARIABLES

Column: 150 \times 4.5 5 μ m Ultrasphere ODS

Mobile phase: MeCN:triethylamine:water:glacial acetic acid 395:5:1585:15

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 4.5

Internal standard: aniline (3.5)

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: alprenolol, betaxolol, caffeine, haloperidol, levobunolol, metoprolol, oxprenolol, pindolol, propranolol

Noninterfering: acetazolamide, atenolol, carbachol, dexamethasone, dipivefrin, epinephrine, gramicidin D, methazolamide, neomycin, pilocarpine, polymyxin B, sulfacetamide, theophylline

Interfering: acebutolol

KEY WORDS

rabbit

REFERENCE

Wu, P.-Y.; Riegel, M.; Ellis, P.P. High-performance liquid chromatographic assay for timolol in the aqueous humor of the eye. *J.Chromatogr.*, **1989**, *494*, 368–375

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Guard column: 40 \times 4.6 SynChropak bulk support (Knauer)

Column: 120 \times 4.6 5 μ m Spherisorb ODS1 C18

Mobile phase: MeCN:MeOH:pH 4.5 acetate buffer (ratio not given)

Flow rate: 1

Detector: UV 294

CHROMATOGRAM**Retention time:** 4.66

OTHER SUBSTANCES**Extracted:** cyclopropane carboxylic acid ester prodrug

KEY WORDSplasma

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 μ m 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:** 4.76**Limit of detection:** <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amoxapine, aspirin, astemizole, atenolol, benzocaine, benzoylecgonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpipramine, carteolol, celiprolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, disopyramide, dosulepine, doxepin, ephedrine, estazolam, etodolac, fenfluramine, fenoprofen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, mephesisin, mephentermine, metapra-

mine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mianserine, midazolam, minoxidil, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nocardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thiopropazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, tiocloamarol, tofisopam, tolbutamide, toloxatone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, vinblastine, warfarin, yohimbine, zopiclone, zorubicine

Interfering: acenocoumarol, alminoprofen, amodiaquine, benazepril, benperidol, chloroquine, cicletanine, cocaine, dipyridamole, doxylamine, droperidol, hydroxychloroquine, ketoprofen, labetalol, meperidine, mepivacaine, mexiletine, moclobemide, nomifensine, pipamperone, pyrimethamine, secobarbital, temazepam, ticlopidine, trazodone, viloxazine, vincristine, vindesine, zolpidem

KEY WORDS

whole blood; plasma

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 μ L MeOH + 500 μ L 500 mM NaOH, vortex for 10 s, add 5 mL dichloromethane:ether 2:3, shake gently for 5 min, centrifuge. Remove the organic layer and evaporate it to dryness at 37° under a stream of nitrogen. Dissolve the residue in 100 μ L 100 mM HCl, add 500 μ L ether, vortex briefly, centrifuge at 3000 rpm for 5 min, inject a 10-25 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 90 \times 4.6 5 μ m Hypersil ODS

Mobile phase: MeCN:buffer 35:65 (Buffer contained 30 mM sodium lauryl sulfate and 50 mM Na₂HPO₄ adjusted to pH 2 with orthophosphoric acid.)

Flow rate: 2

Injection volume: 10-25

Detector: UV 229

CHROMATOGRAM

Retention time: 7

Internal standard: timolol maleate

OTHER SUBSTANCES

Extracted: trimethoprim

KEY WORDS

serum; timolol is IS

REFERENCE

Hung, C.T.; Perrier, D.G. Determination of trimethoprim and sulfamethoxazole in serum by reversed-phase and ion pair HPLC. *J. Liq. Chromatogr.*, **1985**, *8*, 521-536

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 1-2 mL Plasma + 100 μ L 4 M NaOH + 100 μ L 100 μ g/mL metoprolol tartrate in water + 5 mL dichloromethane, shake by hand for 10 s, vortex vigorously for 3 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μ L mobile phase, filter (0.45 μ m), inject a 40-60 μ L aliquot. Urine. 0.1-1 mL Urine + 100 μ L 4 M NaOH + 100 μ L 1 mg/mL metoprolol tartrate in water + 5 mL dichloromethane, vortex vigorously for 1 min, centrifuge at 1500 g for 5 min. Remove the organic layer and add it to 1 mL 100 mM phosphoric acid, vortex for 1 min, centrifuge at 1500 g for 5 min, inject a 40 μ L aliquot of the aqueous phase.

HPLC VARIABLES**Column:** 250 \times 4 Wakosil 5C18 (Wako)**Mobile phase:** MeCN:water:triethylamine 18:81:1, pH adjusted to 3.0 with phosphoric acid**Flow rate:** 1**Injection volume:** 40-60**Detector:** UV 295

CHROMATOGRAM**Retention time:** 7.36**Internal standard:** metoprolol tartrate (8.69)**Limit of detection:** 0.5 ng/mL

OTHER SUBSTANCES**Simultaneous:** N-acetylprocainamide, alprenolol, atenolol, bufetolol, bupranolol, carteolol, disopyramide, indenolol, lidocaine, nicainoprol, nifedipine, pindolol, procainamide, propranolol, quinidine**Noninterfering:** diltiazem, glycinexylidide, mexiletine, nicardipine, tocainide, verapamil**Interfering:** acebutolol

KEY WORDS

plasma

REFERENCEKubota, K.; Nakamura, H.; Koyama, E.; Yamada, T.; Kikuchi, K.; Ishizaki, T. Simple and sensitive determination of timolol in human plasma and urine by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1990**, *533*, 255-263

SAMPLE**Matrix:** blood, urine**Sample preparation:** Plasma. 1 mL Plasma + 100 μ L 4 M NaOH + 0.25 μ g phenacetin + 5 mL MTBE, shake gently for 10 min, centrifuge at 900 g for 5 min. Remove the organic layer and evaporate it to dryness at 40° under reduced pressure, reconstitute the residue in 100 μ L mobile phase, inject a 50-100 μ L aliquot. Urine. 1 mL Urine diluted 10-fold with water + 100 μ L 4 M NaOH + 1 μ g phenacetin + 5 mL MTBE, shake gently for 10 min, centrifuge at 900 g for 5 min. Remove the organic layer and evaporate it to dryness at 40° under reduced pressure, reconstitute the residue in 100 μ L mobile phase, inject a 50-100 μ L aliquot.

HPLC VARIABLES**Guard column:** 50 \times 5 40 μ m Waters reverse-phase guard column material**Column:** 100 \times 5 5 μ m Hypersil 5-ODS**Mobile phase:** MeCN:water 13:87 containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid

Flow rate: 2
Injection volume: 50-100
Detector: UV 295

CHROMATOGRAM

Retention time: 3.7
Internal standard: phenacetin (8.6)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Noninterfering: acebutolol, acetaminophen, atenolol, canrenone, disopyramide, hydralazine, labetalol, lignocaine, nadolol, nifedipine, norverapamil, propranolol, sotalol, verapamil, warfarin

KEY WORDS

plasma

REFERENCE

Lennard, M.S.; Parkin, S. Timolol determination in plasma and urine by high-performance liquid chromatography with ultraviolet detection. *J.Chromatogr.*, **1985**, 338, 249-252

SAMPLE

Matrix: cell lysate
Sample preparation: Sonicate lysed cells for 5 min, filter (0.2 μm PTFE), inject an aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μm LiChrospher 100 RP-18
Column: 250 \times 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 18:82 (Buffer was 0.5% triethylamine adjusted to pH 2.5 with 78% phosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 293

CHROMATOGRAM

Retention time: 7.70
Internal standard: timolol

OTHER SUBSTANCES

Extracted: ofloxacin

KEY WORDS

timolol is IS

REFERENCE

Fresta, M.; Spadaro, A.; Cerniglia, G.; Roperio, I.M.; Puglisi, G.; Furneri, P.M. Intracellular accumulation of ofloxacin-loaded liposomes in human synovial fibroblasts. *Antimicrob.Agents Chemother.*, **1995**, 39, 1372-1375

SAMPLE

Matrix: formulations
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 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: 8.2

OTHER SUBSTANCES

Simultaneous: alprenolol, atenolol, metoprolol, nadolol, oxprenolol, pindolol, practolol, propranolol

Interfering: acebutolol, sotalol

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: perfusate

Sample preparation: 50 μ L Perfusate + 50 μ L pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 μ L 500 μ g/mL methyl p-hydroxybenzoate in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 Cosmosil 5C18-P (Nacalai Tesque)

Mobile phase: MeOH:50 mM NaH₂PO₄ 40:60

Flow rate: 1

Injection volume: 50

Detector: UV 290

CHROMATOGRAM

Internal standard: methyl p-hydroxybenzoate

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 beta-blockers. *Pharm.Res.*, **1995**, *12*, 1146–1150

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot of a 1 mg/mL solution.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Chiralcel OD

Mobile phase: Hexane:isopropanol:diethylamine 90:10:0.1

Flow rate: 0.5

Injection volume: 20

Detector: UV 300

CHROMATOGRAM

Retention time: k' 0.85, 1.07 (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 \times 4.6 12 μ m 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' 2.43

OTHER SUBSTANCES

Also analyzed: acebutolol, 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, pyrillamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, tramazoline, tripeleppamine, 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 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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: 7.04 (A), 3.94 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atro-

pine, 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, fenopropfen, 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, methyl dopa, 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, sulfapyrazole, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimetoprim, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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: 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' 2.77

OTHER SUBSTANCES

Also analyzed: acebutolol, bunitrolol, carazolol, celiprolol, esmolol, mepindolol, metoprolol

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: 50 μ L Solution + 50 μ L pH 7.4 PBS + 100 μ L 500 μ g/mL methyl p-hydroxybenzoate in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 Cosmosil 5C18-P (Nacalai Tesque)

Mobile phase: MeOH:50 mM NaH₂PO₄ 40:60

Flow rate: 1

Injection volume: 50

Detector: UV 290

CHROMATOGRAM

Internal standard: methyl p-hydroxybenzoate

KEY WORDS

buffers; 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

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Chiralcel OD (Daicel)

Mobile phase: Hexane:isopropanol:diethylamine 95:5:0.4

Column temperature: 5

Flow rate: 0.7

Detector: UV 224

CHROMATOGRAM

Retention time: 16.26 ((R)-(+)), 25.40 ((S)-(-))

KEY WORDS

chiral

REFERENCE

Aboul-Enein, H.Y.; Islam, M.R. Direct separation and optimization of timolol enantiomers on a cellulose tris-3,5-dimethylphenylcarbamate high-performance liquid chromatographic chiral stationary phase. *J.Chromatogr.*, **1990**, *511*, 109–114

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 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: 1.94

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, fluopropazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phenomorphine, phenoperidine, phenothiazine, phenoxybenzamine, phen-tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pin-dolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pir-tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, 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, thiorida-zine, thiothixene, thonzylamine, tocinide, tolpropamine, tolycaine, tranlycypromine, tra-zodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylo-metazoline

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 electro-chemical oxidation detection. *J.Chromatogr.*, **1985**, 323, 191–225

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 500 μ L 5 M NaOH + 1 g anhydrous sodium sulfate + 2 mL diethyl ether, shake mechanically for 15 min, centrifuge at 734 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:water 40:60 containing 5 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ adjusted to pH 6.5 with phosphoric acid or 1 M KOH

Column temperature: 30 \pm 0.2

Flow rate: 1.3

Injection volume: 20

Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon cell +1300 mV, d.c. mode, Ag/AgCl reference electrode (At the end of each day clean electrode with MeOH as mobile phase and potential -600 mV for 1 min and +1500 mV for 10 min, repeat 3 times. If necessary, wipe with a tissue wetted with water then a tissue wetted with MeOH.)

CHROMATOGRAM

Retention time: 4.88

Limit of quantitation: 15 ppb

OTHER SUBSTANCES

Extracted: alprenolol, metoprolol, nadolol, oxprenolol

Simultaneous: atenolol

REFERENCE

Maguregui, M.I.; Alonso, R.M.; Jiménez, R.M. High-performance liquid chromatography with amperometric detection applied to the screening of β -blockers in human urine. *J.Chromatogr.B*, **1995**, 674, 85-91

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 \times 4.6 5 μ m Spherisorb cyanopropyl; B 250 \times 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 300

CHROMATOGRAM

Retention time: 11.5

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol

Interfering: acebutolol

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

ANNOTATED BIBLIOGRAPHY

He, H.; Edeki, T.I.; Wood, A.J.J. Determination of low plasma timolol concentrations following topical application of timolol eye drops in humans by high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.B*, **1994**, *661*, 351–356 [LOD 0.1072 ng/mL]

Hermansson, J.; Grahn, A. Resolution of racemic drugs on a new chiral column based on silica-immobilized cellobiohydrolase. Characterization of the basic properties of the column. *J.Chromatogr.*, **1994**, *687*, 45–59 [chiral; also acebutolol, atenolol, betaxolol, bisoprolol, carbutoleol, cathinone, cimetine, dobutamine, dpropizine, epanolol, epinephrine, laudanosine, metanephrine, metoprolol, moprolol, norepinephrine, normetanephrine, octopamine, oxybutynine, pamatolol, practolol, prilocaine, propafenone, proxyphylline, sotalol, talinolol, tetrahydropapaveroline, tetramisole, tolamolol, toliprolol]

Armstrong, D.W.; Chen, S.; Chang, C.; Chang, S. A new approach for the direct resolution of racemic beta adrenergic blocking agents by HPLC. *J.Liq.Chromatogr.*, **1992**, *15*, 545–556 [chiral; also alprenolol, atenolol, cateolol, labetolol, metoprolol, nadolol, oxprenolol, pindolol, propranolol]

Mazzo, D.J.; Snyder, P.A. High-performance liquid chromatography of timolol and potential degradates on dynamically modified silica. *J.Chromatogr.*, **1988**, *438*, 85–92 [simultaneous degradation products; column temp 35; transdermal patches; ophthalmic solutions; tablets]

Gregg, M.R.; Jack, D.B. Determination of timolol in plasma and breast milk using high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1984**, *305*, 244–249

Mazzo, D.J. Simultaneous determination of maleic acid and timolol by high-performance liquid chromatography. *J.Chromatogr.*, **1984**, *299*, 503–507

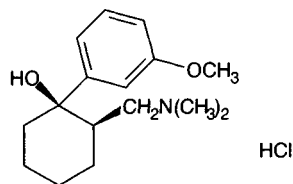
Lefebvre, M.A.; Girault, J.; Fourtillan, J.B. β -Blocking agents: Determination of biological levels using high performance liquid chromatography. *J.Liq.Chromatogr.*, **1981**, *4*, 483–500 [fluorescence detection; plasma; also acebutolol, atenolol, metoprolol, oxprenolol, pindolol, propranolol, sotalol]

Tramadol

Molecular formula: C₁₆H₂₅NO₂

Molecular weight: 263.4

CAS Registry No.: 27203-92-5 (tramadol), 22204-88-2 (tramadol hydrochloride)



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: 11.0

Limit of detection: 6.3 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, butorphanol, codeine, ethylmorphine, fentanyl, morphine, nalbuphine

Noninterfering: atropine, diazepam, neostigmine, pancuronium, succinylcholine, thiopental

Interfering: meperidine

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: Adjust pH of 1 mL urine to >10 with 25% aqueous ammonia, add 4 mL n-hexane, shake for 15 min, centrifuge at 3000 g for 15 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: 25 × 4.6 10 μm Chiralpak AD amylose tris-3,5-dimethylphenyl carbamate

Column: 250 × 4.6 10 μm Chiralpak AD amylose tris-3,5-dimethylphenyl carbamate

Mobile phase: n-Hexane:isopropanol:diethylamine 97.5:2.5:0.01

Flow rate: 1

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 10 (+), 15 (-)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

chiral; pharmacokinetics

REFERENCE

Elsing, B.; Blaschke, G. Achiral and chiral high-performance liquid chromatographic determination of tramadol and its major metabolites in urine after oral administration of racemic tramadol. *J.Chromatogr.*, **1993**, *612*, 223–230

SAMPLE

Matrix: urine

Sample preparation: Adjust pH of 1 mL urine to 9 with 50-100 μL 50 mM pH 10.5 Tris buffer, add 50 μL 50 $\mu\text{g}/\text{mL}$ IS in water, add 4 mL n-hexane:ethyl acetate 80:20, shake for 15 min, centrifuge at 3000 g for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 25 \times 4 5 μm RP Select B (Merck)

Column: 250 \times 4 5 μm RP Select B (Merck)

Mobile phase: MeOH:50 mM pH 3.0 phosphate buffer 30:70

Flow rate: 1

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 12.8

Internal standard: 1-(m-ethoxyphenyl)-2-(dimethylaminomethyl)cyclohexan-1-ol (24.9)

Limit of detection: 80 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pharmacokinetics

REFERENCE

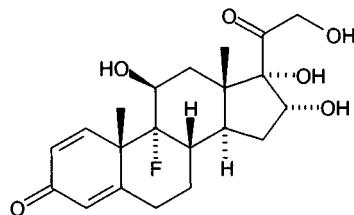
Elsing, B.; Blaschke, G. Achiral and chiral high-performance liquid chromatographic determination of tramadol and its major metabolites in urine after oral administration of racemic tramadol. *J.Chromatogr.*, **1993**, *612*, 223–230

Triamcinolone

Molecular formula: C₂₁H₂₇FO₆

Molecular weight: 394.4

CAS Registry No.: 124-94-7, 76-25-5 (acetone), 1997-15-5 (acetone disodium phosphate), 31002-79-6 (benetone), 5611-51-8 (hexacetone), 67-78-7 (diacetate), 4989-94-0 (furetone), 989-96-8 (21-dihydrogen phosphate)



SAMPLE

Matrix: blood

Sample preparation: Extract plasma with 12 mL dichloromethane, wash the organic layer with 2 mL 100 mM NaOH and 1 mL water, dry the organic layer over 1 g anhydrous sodium sulfate. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb silica

Mobile phase: Hexane:dichloromethane:EtOH:glacial acetic acid 26:69:3.4:2

Flow rate: 0.75

Detector: UV 254

CHROMATOGRAM

Retention time: 6.0 (triamcinolone acetone)

Internal standard: methylprednisolone (13.4)

OTHER SUBSTANCES

Extracted: hydrocortisone

Noninterfering: cortisone

KEY WORDS

plasma; normal phase

REFERENCE

Rohatagi, S.; Hochhaus, G.; Möllmann, J.; Barth, J.; Galia, E.; Erdmann, M.; Sourgens, H.; Derendorf, H. Pharmacokinetic and pharmacodynamic evaluation of triamcinolone acetone after intravenous, oral, and inhaled administration. *J.Clin.Pharmacol.*, **1995**, *35*, 1187–1193

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 NaH₂PO₄ 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; UV 256; UV 343

CHROMATOGRAM

Retention time: 9.59 (triamcinolone)

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, dexamethasone, fluen-drenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17 β -hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE

Volin, P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, 666, 347-353

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 10 μ L 1 μ g/mL fluocinolone acetonide in water + 1.2 mL dichloromethane, shake 1 min, repeat extraction. Combine organic layers and evaporate a 2 mL aliquot under reduced pressure below 40°. Dissolve the residue in 100 μ L MeCN, add 10 μ L reagent 1, add 10 μ L reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μ L water, add 200 μ L MeOH:water 1:1, apply to a Sep-Pak C18 cartridge, wash vial into cartridge with 2 mL MeOH:water 1:1, wash cartridge with 40 mL MeOH:water 1:1, elute with 5 mL MeOH. Concentrate eluate to 500 μ L by evaporation under reduced pressure below 40°, inject 20 μ L aliquot. (Reagent 1 was prepared by dissolving 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, adding 700 mg 4-piperidinopyridine, and diluting to 10 mL with acetonitrile. Reagent 2 was 70 mg/mL 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in MeCN.)

HPLC VARIABLES

Guard column: 50 \times 4.6 7 μ m Zorbax ODS

Column: 250 \times 4.6 7 μ m 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: 43.7 (triamcinolone), 49.4 (triamcinolone acetonide)

Internal standard: fluocinolone acetonide (40.7)

Limit of detection: 0.6 pg/mL; 3 ng/mL (acetonide)

OTHER SUBSTANCES

Extracted: aldosterone, corticosterone, cortisone, dexamethasone, hydrocortisone

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: 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 μ L MeCN:water 30:70, inject whole sample.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: MeCN:water 30:70

Flow rate: 1

Injection volume: 50

Detector: enzyme immunoassay of fractions

CHROMATOGRAM

Retention time: 6 (triamcinolone)

Limit of detection: 0.3 pg

OTHER SUBSTANCES

Extracted: betamethasone, dexamethasone, flumethasone

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: 1 mL Plasma + 5 mL water + 1 mL 2 μ g/mL equilenin in MeOH + 50 μ L 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 μ L mobile phase, inject a 25 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak 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: 3.5 (triamcinolone)

Internal standard: equilenin (7.5)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: betamethasone, deoxycortisol, dexamethasone, hydrocortisone, prednisolone, prednisone

KEY WORDS

Anal. Abs. 1982, 43, 4D182; 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, 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 μ L under a stream of nitrogen at 37°, inject a 5-20 μ L 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 μ L MeOH:water 65:35, inject a 5-20 μ L 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 μ L MeOH:water 65:35, centrifuge, inject a 5-20 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 70 \times 6 35-50 μ m Bondapak C18 Corasil

Column: 250 \times 10 5 μ m 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: 25.95 (triamcinolone), 33.99 (triamcinolone acetonide)

OTHER SUBSTANCES

Extracted: metabolites, cortexolone, cortisol glucuronide, cortisone, hydrocortisone, 6 β -hydroxycortisol

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: Urine. 5 mL Urine + 100 ng prednisone, adjust pH to 7 with 500 mM pH 7 ammonium acetate buffer, extract twice with 7 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L MeOH, inject a 20 μ L aliquot. Serum. 2 mL Serum + 20 ng prednisone, extract twice with 4 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 5 μ m Lichrospher 100 RP-18

Column: 125 \times 4 5 μ m Lichrospher 100 RP-18

Mobile phase: Gradient. A was 10 mM ammonium acetate. B was MeOH. A:B from 50:50 to 0:100 over 7 min, maintain at 0:100 for 3 min.

Flow rate: 1

Injection volume: 20

Detector: MS, PE Sciex API III LC-MS-MS, heated nebulizer interface at 75 psi and 300°, auxiliary nitrogen 1.4 L/min, nitrogen curtain 1.2 L/min, negative mode ionization, discharge current -3 μ A, collision gas argon with 570×10^{12} molecules/sq.cm., dwell time 10 ms, step size 1 amu, m/z 413, SIM

CHROMATOGRAM

Retention time: 8 (triamcinolone acetonide)

Internal standard: prednisone (m/z 327) (5.5)

Limit of detection: 0.1 ng/mL

KEY WORDS

horse; serum; pharmacokinetics

REFERENCE

Koupai-Abyazani, M.R.; Yu, N.; Esaw, B.; Laviolette, B. Determination of triamcinolone acetonide in equine serum and urine by liquid chromatography-atmospheric pressure ionization mass spectrometry. *J.Anal.Toxicol.*, **1995**, *19*, 182–186

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 2.5 mg/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Lichrosorb Si-100

Mobile phase: Chloroform (stabilized with amylene):MeOH:water 978.6:20:1.4

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 6.4 (triamcinolone acetonide)

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

normal phase

REFERENCE

Cavina, G.; Alimenti, R.; Gallinella, B.; Valvo, L. The identification of related substances in triamcinolone acetonide by means of high-performance liquid chromatography with diode array detector and mass spectrometry. *J.Pharm.Biomed.Anal.*, **1992**, *10*, 685–692

SAMPLE**Matrix:** bulk**Sample preparation:** Prepare a 2.5 mg/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Spherisorb ODS**Mobile phase:** MeCN:water 36:64**Flow rate:** 1**Injection volume:** 20**Detector:** UV 240

CHROMATOGRAM**Retention time:** 6.4 (triamcinolone acetonide)

OTHER SUBSTANCES**Simultaneous:** impurities

REFERENCE

Cavina, G.; Alimenti, R.; Gallinella, B.; Valvo, L. The identification of related substances in triamcinolone acetonide by means of high-performance liquid chromatography with diode array detector and mass spectrometry. *J.Pharm.Biomed.Anal.*, **1992**, *10*, 685-692

SAMPLE**Matrix:** formulations**Sample preparation:** Weigh out amount containing 10 mg triamcinolone acetonide, make up to 50 mL with mobile phase. Remove a 2 mL aliquot and dilute it to 10 mL with mobile phase, filter, inject a 20 μ L aliquot of the filtrate.

HPLC VARIABLES**Column:** 125 \times 4 5 μ m LiChrospher 100 RP-18**Mobile phase:** MeOH:water:96% acetic acid 55:44:1, pH 3.0**Flow rate:** 1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5.02 (triamcinolone acetonide)

OTHER SUBSTANCES**Simultaneous:** salicylic acid

KEY WORDS

topical solution

REFERENCE

Kedor-Hackmann, E.R.M.; Gianotto, E.A.S.; Santoro, M.I.R.M. Determination of triamcinolone acetonide and salicylic acid in pharmaceutical formulations by high performance liquid chromatography. *Pharmazie*, **1996**, *51*, 63

SAMPLE**Matrix:** formulations**Sample preparation:** Dermatological patch (2 cm \times 2 cm) + 2 mL hexane, shake mechanically for 10 min, add 8 mL mobile phase, mix thoroughly, centrifuge at 2500 rpm for 10 min, remove 1 mL of lower phase, inject a 10 μ L aliquot of this solution.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb C8

Mobile phase: MeOH:water 70:30

Flow rate: 0.5

Injection volume: 10

Detector: UV 240

KEY WORDS

Chem.Abs., 114, 129259; dermatological patches; stability-indicating; for triamcinolone acetonide

REFERENCE

Edwardson, P.A.D.; Gardner, R.S. Problems associated with the extraction and analysis of triamcinolone acetonide in dermatological patches. *J.Pharm.Biomed.Anal.*, **1990**, 8, 935–938

SAMPLE

Matrix: formulations, solutions

Sample preparation: Ointment. 1 g Ointment + 5 mL MeOH + 5 mL water + 800 μL 1 mg/mL hydrocortisone in EtOH, stir until a clear solution forms, make up to 25 mL with water, inject a 20 μL aliquot. Solutions. 8 mL Solution + 800 μL 1 mg/mL hydrocortisone in EtOH + 5 mL MeOH, make up to 25 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 μm Bondapak C18

Mobile phase: MeCN:200 mM KH₂PO₄ 32:68, pH 4.2

Flow rate: 3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8 (triamcinolone acetonide)

Internal standard: hydrocortisone (4)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

ointment; stability-indicating

REFERENCE

Das Gupta, V. Stability of triamcinolone acetonide solutions as determined by high-performance liquid chromatography. *J.Pharm.Sci.*, **1983**, 72, 1453–1456

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 mm long 5 μm Microsorb-MV C18

Mobile phase: MeCN:water 60:40

Flow rate: 1

Detector: UV 240

KEY WORDS

for triamcinolone acetonide

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: 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, bromazepam, 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, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, 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, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phen-suximide, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, 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, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

KEY WORDS

for triamcinolone

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 10-60 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 2.5 μ m Spherisorb ODS1

Mobile phase: MeCN:water 50:50

Flow rate: 0.16-0.30

Injection volume: 10-60

Detector: UV 240

CHROMATOGRAM

Retention time: 17 (triamcinolone acetonide)

OTHER SUBSTANCES

Simultaneous: halcinonide

KEY WORDS

Chem.Abs., 114, 136153

REFERENCE

Gardner, R.S.; Walker, M.; Hollingsbee, D.A. A sensitive high-performance liquid chromatographic method for the assessment of percutaneous absorption of topical corticosteroids. *J.Pharm. Biomed.Anal.*, **1990**, *8*, 1083-1085

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Phenyl (Waters) or 12% ODS (Whatman)

Mobile phase: MeCN:water 30:70

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 10.1 (phenyl) or 15.0 (ODS) (triamcinolone acetonide)

OTHER SUBSTANCES

Simultaneous: fluoxymesterone

KEY WORDS

Chem.Abs., 98, 166972; Proceedings of the Symposium on the Analysis of Steroids, Eger, Hungary, 1981

REFERENCE

Kirschbaum, J.; Clay, R.; Poet, R. HPLC steroid analyses: generic column description and variable selectivity. *Anal.Chem.Symp.Ser. (Adv.Steroid Anal.)*, **1982**, 10, 361-366

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: μ Bondapak ODS

Mobile phase: MeCN:water 30:70

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 12 (triamcinolone acetonide)

Internal standard: fluoxymesterone (10)

REFERENCE

Kirschbaum, J. High-pressure liquid chromatography of triamcinolone acetonide: effect of different octadecylsilane columns on mobility. *J.Pharm.Sci.*, **1980**, 69, 481-482

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 \times 21 μ m 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 μ L 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 μ L 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 \times 4.6 5 μ m 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; MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM

Retention time: 10 (triamcinolone acetonide)

Limit of detection: 100 ppb

OTHER SUBSTANCES

Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, trenbolone, 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.Chrom.Rel.Technol.*, **1996**, *19*, 69–87

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na₂HPO₄, 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: 2.9 (triamcinolone), 14 (triamcinolone acetonide)

Internal standard: methylprednisolone (9)

OTHER SUBSTANCES

Extracted: betamethasone, corticosterone, cortisone, dexamethasone, fluorocortisone acetate, fluorocortisone, hydrocortisone, hydroxyprogesterone, prednisolone, prednisone

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 + 1.5 µg betamethasone + 100 mg K₂HPO₄ + 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: 60 × 4.6 3 µm Hypersil ODS

Mobile phase: Gradient. MeOH:150 mM ammonium acetate from 40:60 to 50:50 over 6 min, maintain at 50:50 for 1 min, to 60:40 over 3 min, maintain at 60:40 for 5 min

Flow rate: 0.8

Injection volume: 15

Detector: MS, Hewlett-Packard HP 5988A, vaporizer probe 92° decreased to 89° over 6 min, decreased to 86° over 3 min, maintain at 86° for 5 min, ion source 276°, emission current 150 µA, electron energy 955 eV, positive ion mode, filament on

CHROMATOGRAM**Retention time:** 2.7 (triamcinolone), 7.5 (triamcinolone acetoneide)**Internal standard:** betamethasone (7)**Limit of detection:** 1-5 ng (SIM)

OTHER SUBSTANCES**Extracted:** corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 11 α -hydroxyprogesterone, prednisolone, prednisone

REFERENCEPark, 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 + 1.5 μ g betamethasone + 100 mg K₂HPO₄ + 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 μ L MeOH, filter (0.45 μ m), inject a 15 μ L aliquot.

HPLC VARIABLES**Column:** 100 \times 4.6 5 μ m 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:** 9.19 (triamcinolone), 13.82 (triamcinolone acetoneide)**Internal standard:** betamethasone**Limit of detection:** 10 ng/mL

OTHER SUBSTANCES**Extracted:** corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 11 α -hydroxyprogesterone, prednisolone, prednisone

REFERENCEPark, 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

ANNOTATED BIBLIOGRAPHYValvo, L.; Paris, A.; Savella, A.L.; Gallinella, B.; Ciranni Signoretti, E. General high-performance liquid chromatographic procedures for the rapid screening of natural and synthetic corticosteroids. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 805-810 [gradient; reverse phase; normal phase; also beclomethasone, beclomethasone 17,21-dipropionate, betamethasone, betamethasone 21-acetate, betamethasone 17,21-dipropionate, betamethasone 21-disodium phosphate, betamethasone 17-valerate, cortisone, cortisone 21-acetate, 11-deoxycorticosterone 21-acetate, dexamethasone, dexamethasone 21-acetate, dexamethasone 21-disodium phosphate, fluocinolone, fluocinolone acetoneide, 9 α -fluorohydrocortisone 21-acetate, 9 α -fluorohydrocortisone, 9 α -fluoroprednisolone, 9 α -fluoroprednisolone 21-acetate, hydrocortisone, hydrocortisone 21-acetate, hydrocortisone 21-hemisuccinate, 6 α -methylprednisolone, 6 α -methylprednisolone 21-acetate, 6 α -methylprednisolone 21-sodium succinate, prednisolone, prednisolone 21-acetate, prednisolone 21-disodium phosphate, prednisolone 21-pivalate, prednisolone 21-sodium succinate, prednisone; for triamcinolone and triamcinolone acetoneide]

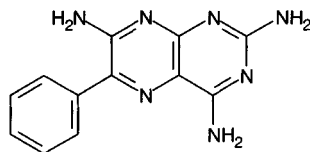
- Santos-Montes, A.; Gasco-Lopez, A.I.; Izquierdo-Hornillos, R. Optimization of the high-performance liquid chromatographic separation of a mixture of natural and synthetic corticosteroids. *J.Chromatogr.*, **1993**, *620*, 15–23 [simultaneous betamethasone, corticosterone, cortisone, deoxycorticosterone, dexamethasone, fluorocortisone, hydrocortisone, hydroxyprogesterone, methylprednisolone, prednisolone, prednisone; for triamcinolone]
- Rowland, J.M.; Althaus, Z.R.; Slikker, W., Jr.; Hendrickx, A.G. Comparative distribution and metabolism of triamcinolone acetonide and cortisol in the rat embryomaternal unit. *Teratology*, **1983**, *27*, 333–341 [rat; plasma; tissue; gradient; for triamcinolone and triamcinolone acetonide; extracted cortisone, hydrocortisone]
- Schöneshöfer, M.; Kage, A.; Weber, B. New "on-line" sample-pretreatment procedure for routine liquid-chromatographic assay of low-concentration compounds in body fluids, illustrated by triamcinolone assay. *Clin.Chem.*, **1983**, *29*, 1367–1371 [column-switching; LOD 5 ng/mL; urine; for triamcinolone]
- Gordon, G.; Wood, P.R. Use of reversed-phase high-performance liquid chromatography in the analysis of products containing halcinonide and triamcinolone acetonide. *Proc.Anal.Div.Chem.Soc.*, **1977**, *14*, 30–32
- Gordon, G.; Wood, P.R. Determination of triamcinolone acetonide in cream and suspension formulations by high-performance liquid chromatography. *Analyst*, **1976**, *101*, 876–882 [simultaneous excipients, prednisolone; for triamcinolone acetonide]

Triamterene

Molecular formula: C₁₂H₁₁N₇

Molecular weight: 253.3

CAS Registry No.: 396-01-0



SAMPLE

Matrix: bile, blood, urine

Sample preparation: 1 mL Plasma, whole blood, bile, or urine + 100 μ L 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 μ L mobile phase, inject a 20 μ L aliquot. (Hydrolyze conjugates as follows. 500 μ L Plasma, whole blood, bile, or urine + 500 μ L pH 5 buffer + 50 μ L glucosylase, heat at 50° for 24 h, add 50 μ L 1 M NaOH, add 100 μ L 1 μ g/mL triamterene in MeOH, add 1 mL 100 mM sodium bicarbonate, proceed as above. In order to determine only the sulfate add 60 μ L neutralized 100 mM 1,4-saccharolactone solution to inhibit β -glucuronidase.)

HPLC VARIABLES

Guard column: 25 \times 2.3 5 μ m PRP-1 (Hamilton)

Column: 150 \times 4.1 5 μ m 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: 10

Internal standard: triamterene

OTHER SUBSTANCES

Extracted: dilevalol

Noninterfering: acebutolol, brefanolol, carteolol, carvedilol, enalapril, furosemide, hydrochlorothiazide, isosorbide dinitrate, metoprolol, nifedipine, pirtanide, propranolol, verapamil, xamoterol, xipamide

KEY WORDS

plasma; whole blood; triamterene is IS; 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: 100 μ L Plasma or whole blood + 400 μ L 3.5 μ g/mL furosemide in MeCN, vortex for 1 min, centrifuge at 3200 rpm for 10 min. Remove the supernatant and evaporate it to 200 μ L under a stream of nitrogen, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m Micro Pak MCH reverse phase

Mobile phase: MeCN:0.02% phosphoric acid 30:70 adjusted to pH 4.0 with 500 mM NaOH

Flow rate: 2

Detector: F ex 365 em 440

CHROMATOGRAM

Retention time: 5.7

Internal standard: furosemide (8.1)

Limit of quantitation: 1 ng/mL

KEY WORDS

whole blood; plasma; pharmacokinetics

REFERENCE

Sörgel, F.; Lin, E.T.; Hasegawa, J.; Benet, L.Z. Liquid chromatographic analysis of triamterene and its major metabolite, hydroxytriamterene sulfate, in blood, plasma, and urine. *J.Pharm.Sci.*, **1984**, *73*, 831-833

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Dilute 200 μ L plasma with 600 μ L water, inject a 20 μ L aliquot. Urine. Dilute 10 μ L urine with 2 mL water, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.5 μ m Spherisorb-amino

Mobile phase: Buffer containing 10 mM perchloric acid, 2 mM triethylamine, and 100 mM ammonium acetate

Flow rate: 3

Injection volume: 20

Detector: F ex 360 em 436

CHROMATOGRAM

Retention time: 0.5

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: hydroxytriamterene sulfate

Noninterfering: bendroflumethiazide, butizide, chlorthalidone, furosemide, hydrochlorothiazide, hydroxytriamterene, nifedipine

KEY WORDS

plasma

REFERENCE

Mascher, H.; Wasilewski, M. Simple and fast HPLC determination of triamterene and hydroxytriamterenesulphate in plasma and urine. *J.Liq.Chromatogr.*, **1994**, *17*, 1577-1585

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 150 μ L Plasma + 10 μ L 70% perchloric acid, shake for 15 min, centrifuge at 900 g for 10 min, inject 30 μ L of the supernatant. Urine. Dilute urine with 2 volumes of MeOH, centrifuge at 900 g for 10 min, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Nova-Pak C18

Mobile phase: MeCN:MeOH:buffer 14:8:70 (Buffer obtained from 6.75 mL 89% phosphoric acid in 900 mL water, pH adjusted to 2.8 with triethylamine, volume made up to 1 L.)

Flow rate: 0.8

Injection volume: 5-30

Detector: F ex 340-380 em 400-600

CHROMATOGRAM

Retention time: 3.3

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

plasma

REFERENCE

Swart, K.J.; Botha, H. Rapid method for the determination of the diuretic triamterene and its metabolites in plasma and urine by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, *413*, 315-319

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute 100 μ L urine with 1 mL water. 1 mL Plasma or diluted urine + 40 μ L 1 μ g/mL p-methoxytriamterene in MeOH + 200 mg sodium bicarbonate + 5 mL ethyl acetate, shake for 1 min, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, wash sides of tube with ethyl acetate and again evaporate to dryness, dissolve residue in 20-50 μ L MeOH, inject a 5-12 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeOH:0.1% KH₂PO₄ 45:55, final pH adjusted to 3.8 with phosphoric acid

Flow rate: 2

Injection volume: 5-12

Detector: F ex 365 em 440; UV 230

CHROMATOGRAM

Retention time: 2.6

Internal standard: p-methoxytriamterene (3.1)

Limit of detection: 1 ng/mL (F); 10 ng/mL (UV)

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma

REFERENCE

Brodie, R.R.; Chasseaud, L.F.; Taylor, T.; Walmsley, L.M. Determination of the diuretic triamterene in the plasma and urine of humans by high-performance liquid chromatography. *J.Chromatogr.*, **1979**, *164*, 527-533

SAMPLE

Matrix: formulations, urine

Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 30 min, sonicate for 5 min, filter (Albet 242 paper), wash solid with MeOH, make up filtrate to 50 mL with MeOH, inject a 20 μ L aliquot. Urine. Adjust pH of 2 mL urine to 10.0 with 2 M KOH, add 1.5 mg NaCl, add 4 mL ethyl acetate, shake for 10 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 2 mL mobile phase, sonicate, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeCN:water 30:70 containing 5 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH adjusted to 5.5

Flow rate: 1

Injection volume: 20

Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1300 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 5 min.)

CHROMATOGRAM

Retention time: 5.01

Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: furosemide

KEY WORDS

tablets; pharmacokinetics

REFERENCE

Barroso, M.B.; Alonso, R.M.; Jiménez, R.M. Simultaneous determination of the diuretics triamterene and furosemide in pharmaceutical formulations and urine by HPLC-EC. *J.Liq.Chrom.Rel.Technol.*, 1996, 19, 231-246

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4 5 μ m 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: 6.73 (A), 3.90 (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, fenopropfen, 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, methyl dopa, 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, pimizide, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thietilperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 10 μ L aliquot of an aqueous solution.

HPLC VARIABLES

Column: 250 \times 4.5 μ m Lichrosphere CN

Mobile phase: 2% acetic acid

Flow rate: 1

Injection volume: 10

Detector: UV 320

CHROMATOGRAM

Retention time: 12.8

OTHER SUBSTANCES

Simultaneous: caffeic acid

REFERENCE

Uang, Y.-S.; Kang, F.-L.; Hsu, K.-Y. Determination of caffeic acid in rabbit plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, 673, 43–49

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 KH₂PO₄ and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 211; UV 245

CHROMATOGRAM

Retention time: 1.6

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, 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: urine

Sample preparation: Inject an aliquot of urine directly onto column A with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B.

HPLC VARIABLES

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

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 NaH₂PO₄ + 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: 6.9

Limit of detection: 7 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, furosemide, hydrochlorothiazide, probenecid, spironolactone

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

SAMPLE

Matrix: urine

Sample preparation: Inject 5 μL urine onto column A in mobile phase A, after 1 min elute the contents of column A onto column B with mobile phase B

HPLC VARIABLES

Column: A 20 \times 2.1 30 μm Hypersil ODS-C18; B 125 \times 4 5 μm HP-LiChrospher 100 RP 18

Mobile phase: A water; B Gradient. A was MeCN. B was 3.45 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ + 700 μL propylamine hydrochloride in 500 mL water, pH adjusted to 3 with concentrated phosphoric acid. A:B from 0:100 to 50:50 after 4 min then hold at 50:50.

Flow rate: 1

Injection volume: 5

Detector: F ex 230 em 430

CHROMATOGRAM

Retention time: 5.27

Limit of detection: 5 ng/mL

KEY WORDS

column-switching

REFERENCE

Campíns-Falcó, P.; Herráez-Hernández, R.; Sevillano-Cabeza, A. Determination of triamterene in urine by HPLC using fluorescence detection and column-switching. *Chromatographia*, **1994**, *38*, 29–34

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 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 \times 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 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ + 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: 3.8

Internal standard: triamterene

OTHER SUBSTANCES

Extracted: chlorthalidone

Simultaneous: atenolol, oprenolol, reserpine, spironolactone

KEY WORDS

SPE; triamterene is IS

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 μ L 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 \times 4.5 μ m Hypersil octadecylsilica ODS; B 200 \times 4.6 5 μ m 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 NaH₂PO₄·H₂O in 1 L water, pH adjusted to 3.1 with phosphoric acid.)**Flow rate:** 1**Injection volume:** 40**Detector:** UV 360

CHROMATOGRAM**Retention time:** 11.6**Limit of detection:** 500 ng/mL

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

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCESaarinen, 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 μ L 100 μ g/mL 7-propyltheophylline in MeOH + 200 μ L 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 μ L MeCN:water 15:85 and inject 20 μ L 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 \times 4.6 3 μ m Ultrasphere ODS**Mobile phase:** Gradient. MeCN:buffer 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, to 10:90 over 1 min, equilibrate at 10:90 for 2 min. (Buffer was 100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid.)**Flow rate:** 1**Injection volume:** 20**Detector:** UV 270

CHROMATOGRAM**Retention time:** 3.7**Internal standard:** 7-propyltheophylline (4.5)

OTHER SUBSTANCES

Simultaneous: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, 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: 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 μ L MeCN/water, inject a 10-20 μ L aliquot.

HPLC VARIABLES**Column:** 100 \times 4.5 μ m 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; UV 270

CHROMATOGRAM**Retention time:** 4.0**Limit of detection:** 50 ng (by MS)

OTHER SUBSTANCES

Extracted: amiloride, bendroflumethiazide, benzthiazide, chlorthalidone, furosemide

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 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 μ L 50 μ g/mL β -hydroxyethyltheophylline in MeOH, inject 5 μ L aliquot. (Solid buffer I was $\text{KH}_2\text{PO}_4:\text{Na}_2\text{HPO}_4$ 99:1, solid buffer II was $\text{NaHCO}_3:\text{K}_2\text{CO}_3$ 3:2.)

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m 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 NaH₂PO₄ 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: 6.8 (A), 7.6 (B)

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

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

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

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

Interfering: flumethiazide

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: 20-100 μ L Urine + 1 mL 500 μ g/mL hydroflumethiazide in MeCN, vortex, centrifuge. Remove the supernatant, inject a 2 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m Micro Pak MCH reverse phase

Mobile phase: MeCN:0.02% phosphoric acid 13:87 adjusted to pH 5.3 with NaOH

Flow rate: 2

Injection volume: 2

Detector: F ex 365 em 440

CHROMATOGRAM

Retention time: 8.6

Internal standard: hydroflumethiazide (7.0)

Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pharmacokinetics

REFERENCE

Sörgel, F.; Lin, E.T.; Hasegawa, J.; Benet, L.Z. Liquid chromatographic analysis of triamterene and its major metabolite, hydroxytriamterene sulfate, in blood, plasma, and urine. *J.Pharm.Sci.*, **1984**, *73*, 831–833

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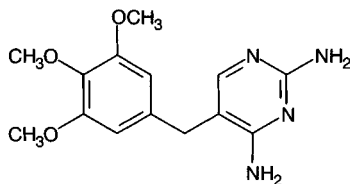
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- Bonet-Domingo, E.; Medina-Hernandez, M.J.; Garcia-Alvarez-Coque, M.C. A micellar liquid chromatographic procedure for the determination of amiloride, bendroflumethiazide, chlorthalidone, spironolactone and triamterene in pharmaceuticals. *J.Pharm.Biomed.Anal.*, **1993**, *11*, 711–716
- Herráez-Hernández, R.; Campíns-Falcó, P.; Sevillano-Cabeza, A. Improved screening procedure for diuretics. *J.Liq.Chromatogr.*, **1992**, *15*, 2205–2224 [LOD 10-1000 ng/mL; gradient; urine; hydroxymethyltheophylline (IS); extracted acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, furosemide, hydrochlorothiazide, probenecid, spironolactone]
- Campíns-Falcó, P.; Herráez-Hernández, R.; Sevillano-Cabeza, A. Solid-phase extraction techniques for assay of diuretics in human urine samples. *J.Liq.Chromatogr.*, **1991**, *14*, 3575–3590 [SPE; hydroxymethyltheophylline (IS); extracted acetazolamide, amiloride, bendroflumethiazide, bumetanide, chlorthalidone, cyclothiazide, ethacrynic acid, furosemide, hydrochlorothiazide, probenecid, spironolactone]
- Shah, V.P.; Walker, M.A.; Prasad, V.K. Application of flow programming in the analysis of drugs and their metabolites in biological fluids. *J.Liq.Chromatogr.*, **1983**, *6*, 1949–1954 [urine; plasma; also chlorothiazide, hydrochlorothiazide]
- Hasegawa, J.; Lin, E.T.; Williams, R.L.; Sörgel, F.; Benet, L.Z. Pharmacokinetics of triamterene and its metabolite in man. *J.Pharmacokinet.Biopharm.*, **1982**, *10*, 507–523 [plasma; urine; fluorescence detection; furosemide (IS); SPE]
- Menon, G.N.; White, L.B. Simultaneous determination of hydrochlorothiazide and triamterene in capsule formulations by high-performance liquid chromatography. *J.Pharm.Sci.*, **1981**, *70*, 1083–1085 [m-hydroxyacetophenone (IS); stability-indicating; simultaneous degradation products]
- Yakatan, G.J.; Cruz, J.E. High-performance liquid chromatographic analysis of triamterene and p-hydroxytriamterene in plasma. *J.Pharm.Sci.*, **1981**, *70*, 949–951 [extracted metabolites; LOD 20 ng/mL]

Trimethoprim

Molecular formula: C₁₄H₁₈N₄O₃

Molecular weight: 290.3

CAS Registry No.: 738-70-5, 56585-33-2 (sulfate)



SAMPLE

Matrix: blood

Sample preparation: 50 μ L Serum + 75 μ L acetone:10% trichloroacetic acid 1:2, vortex for 5 s, centrifuge for 4 min. Remove 62.5 μ L of the supernatant and add it to 62.5 μ L 50 mM KH₂PO₄, add 250 μ L diethyl ether, vortex for 10 s, centrifuge for 5 min, filter (0.45 μ m) the lower aqueous layer, inject a 20 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 75 \times 4.6 TSK gel ODS-80TM (Tosoh)

Mobile phase: MeCN:50 mM pH 6.0 KH₂PO₄ 8:92

Flow rate: 1

Injection volume: 20

Detector: UV 235

CHROMATOGRAM

Retention time: 30

Internal standard: trimethoprim

OTHER SUBSTANCES

Extracted: vancomycin

KEY WORDS

serum; trimethoprim is IS

REFERENCE

Morishige, H.; Shuto, H.; Ieiri, I.; Otsubo, K.; Oishi, R. Instability of standard calibrators may be involved in overestimating vancomycin concentrations determined by fluorescence polarization immunoassay. *Ther. Drug Monit.*, **1996**, *18*, 80-85

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bakerbond C18 SPE cartridge with MeOH and 50 mM pH 5.5 citrate buffer. 500 μ L Serum + 17 μ L 600 μ g/mL sulfamethazine in MeCN: water 20:80 + 500 μ L 50 mM pH 5.5 citrate buffer, vortex, add to the SPE cartridge, wash twice with 50 mM pH 5.5 citrate buffer, air dry, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45 $^{\circ}$, reconstitute the residue in 250 μ L mobile phase, inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 Guard-Pak

Column: 300 \times 3.9 μ Bondapak C18

Mobile phase: MeCN:1% acetic acid 18:82

Flow rate: 2.5

Injection volume: 100

Detector: UV 240

CHROMATOGRAM**Retention time:** 3.14**Internal standard:** sulfamethazine (4.19)

OTHER SUBSTANCES**Extracted:** sulfamethoxazole

KEY WORDSserum; SPE

REFERENCE

Moore, K.H.P.; Brouwer, K.L.R. High-performance liquid chromatographic evaluation of the effect of heat treatment on trimethoprim and sulfamethoxazole stability in serum. *Ther.Drug Monit.*, **1995**, *17*, 356–360

SAMPLE**Matrix:** blood

Sample preparation: Condition a 3 mL 500 mg Sep-Pak with 10 mL MeOH and 10 mL water at 3 mL/min (do not allow to dry). 5 mL Serum + 50 μ L 10 μ g/mL ormetoprim in water, vortex for 15 s, allow to stand for 5 min, add 2 mL pH 11 NaH_2PO_4 (pH adjusted with 5 M NaOH), vortex for 10 s, add 30 mL dichloromethane, shake for 10 min, extract aqueous phase again with 20 mL dichloromethane. Combine organic layers and shake them with 10 mL 150 mM sulfuric acid. Remove 8 mL of the aqueous layer and add it to 10 mL phosphate buffer (adjusted to pH 6 with 1 M NaOH), add this to the SPE cartridge, wash with 10 mL water, draw air through the cartridge for 1 min, elute with 1 mL MeOH, evaporate the eluate at 50° under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, filter (0.45 μ m), inject a 50 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.5 μ m Inertsil C8**Mobile phase:** MeCN:pH 3 ammonium formate-trifluoroacetic acid 20:80**Flow rate:** 1**Injection volume:** 50**Detector:** MS, VG Trio 2 with a thermospray-plasma spray LC interface, capillary probe tip at 300°

CHROMATOGRAM**Internal standard:** ormetoprim

KEY WORDSserum; cow; SPE; thermospray; plasma spray

REFERENCE

Nachilobe, P.; Boison, J.O.; Cassidy, R.M.; Fesser, A.C.E. Determination of trimethoprim in bovine serum by high-performance liquid chromatography with confirmation by thermospray liquid chromatography-mass spectrometry. *J.Chromatogr.*, **1993**, *616*, 243–252

SAMPLE**Matrix:** blood

Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 10 mL MeOH and 10 mL water at 3 mL/min (do not allow to dry). 5 mL Serum + 50 μ L 10 μ g/mL ormetoprim in water, vortex for 15 s, allow to stand for 5 min, add 2 mL pH 11 NaH_2PO_4 (pH adjusted with 5 M NaOH), vortex for 10 s, add 30 mL dichloromethane, shake for 10 min, extract aqueous phase again with 20 mL dichloromethane. Combine organic layers and shake them with 10 mL 150 mM sulfuric acid. Remove 8 mL of the aqueous layer

and add it to 10 mL phosphate buffer (adjusted to pH 6 with 1 M NaOH), add this to the SPE cartridge, wash with 10 mL water, draw air through the cartridge for 1 min, elute with 1 mL MeOH, evaporate the eluate at 50° under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, filter (0.45 µm), inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: C18 pre-column filter

Column: 250 × 4.6 5 µm Spherisorb ODS(2)

Mobile phase: MeCN:MeOH:buffer 150:50:800 (Buffer was 2.5 mL triethylamine in 900 mL water, add 5 mL glacial acetic acid, make up to 1 L with water.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 7.5

Internal standard: ormetoprim (10)

Limit of detection: 5 ng/mL

KEY WORDS

serum; cow; SPE

REFERENCE

Nachilobe, P.; Boison, J.O.; Cassidy, R.M.; Fesser, A.C.E. Determination of trimethoprim in bovine serum by high-performance liquid chromatography with confirmation by thermospray liquid chromatography-mass spectrometry. *J.Chromatogr.*, **1993**, *616*, 243–252

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL C18 Bond Elut SPE cartridge with 500 µL MeOH then 1 mL wash solution. 500 µL Serum + 500 µL wash solution, vortex for 3 s, add 25 µL 240 µg/mL p-nitrophenol in MeOH, vortex for 3 s, add to the SPE cartridge, wash with 1 mL wash solution, dry with vacuum applied for 30 s, elute with two 500 µL aliquots of MeOH:triethylamine 10:1. Combine the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute in 200 µL mobile phase, inject a 50-100 µL aliquot. (Wash solution was 7 mL 3 M HCl + 1.6 g 1-octanesulfonic acid + 150 mL 100 mM disodium citrate made up to 800 mL with water, pH adjusted to 3.00 with 3 M HCl.)

HPLC VARIABLES

Guard column: 20 × 2 40 µm Upchurch pellicular C18

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeCN:buffer 24:76 with 0.8 g/L 1-octanesulfonic acid (Buffer was 7 mL 3 M HCl plus 150 mL 100 mM disodium citrate made up to 760 mL with water, pH adjusted to 3.00 with 3 M HCl.)

Flow rate: 1.5

Injection volume: 50-100

Detector: UV 230

CHROMATOGRAM

Retention time: 5.6

Internal standard: p-nitrophenol (9.3)

Limit of quantitation: 25 ng

OTHER SUBSTANCES

Simultaneous: N⁴-acetylsulfamethoxazole, sulfamethoxazole

KEY WORDS

serum; SPE

REFERENCE

Laizure, S.C.; Holden, C.L.; Stevens, R.C. Ion-paired high-performance liquid chromatographic separation of trimethoprim, sulfamethoxazole and N⁴-acetylsulfamethoxazole with solid-phase extraction. *J.Chromatogr.*, **1990**, 528, 235–242

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Plasma + 100 μ L 5 μ g/mL sulfafurazole in MeOH:water 50:50 + 200 μ L 1 M pH 6.8 sodium phosphate buffer + 6 mL ethyl acetate, shake for 15 min, centrifuge at 600 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 175 μ L MeOH:water 20:80, vortex for 1 min, add 25 μ L 40% trichloroacetic acid in 100 mM hydrochloric acid, vortex for 30 s, centrifuge at 8300 g for 5 min, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES**Column:** 250 \times 4.5 μ m LiChrosorb RP18**Mobile phase:** MeCN:150 mM pH 4.85 ammonium phosphate buffer 12.6:87.4**Flow rate:** 1.5**Injection volume:** 100**Detector:** UV 254

CHROMATOGRAM**Retention time:** 10**Internal standard:** sulfafurazole (21)**Limit of detection:** 160 ng/mL

OTHER SUBSTANCES**Extracted:** N-acetylsulfamethoxazole, sulfamethoxazole

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Van der Steuijt, K.; Sonneveld, P. Concurrent analysis of methotrexate, trimethoprim, sulfamethoxazole and their major metabolites in plasma by high-performance liquid chromatography. *J.Chromatogr.*, **1987**, 422, 328–333

SAMPLE**Matrix:** blood

Sample preparation: 1 mL Serum + 100 μ L 40 μ g/mL timolol maleate in MeOH + 500 μ L 500 mM NaOH, vortex for 10 s, add 5 mL dichloromethane:ether 2:3, shake gently for 5 min, centrifuge. Remove the organic layer and evaporate it to dryness at 37° under a stream of nitrogen. Dissolve the residue in 100 μ L 100 mM HCl, add 500 μ L ether, vortex briefly, centrifuge at 3000 rpm for 5 min, inject a 10–25 μ L aliquot of the aqueous phase.

HPLC VARIABLES**Column:** 90 \times 4.6 5 μ m Hypersil ODS**Mobile phase:** MeCN:buffer 35:65 (Buffer contained 30 mM sodium lauryl sulfate and 50 mM Na₂HPO₄ adjusted to pH 2 with orthophosphoric acid.)**Flow rate:** 2

Injection volume: 10-25

Detector: UV 229

CHROMATOGRAM

Retention time: 3

Internal standard: timolol maleate (7)

Limit of quantitation: 10 ng/mL

KEY WORDS

serum

REFERENCE

Hung, C.T.; Perrier, D.G. Determination of trimethoprim and sulfamethoxazole in serum by reversed-phase and ion pair HPLC. *J.Liq.Chromatogr.*, **1985**, 8, 521-536

SAMPLE

Matrix: blood

Sample preparation: 250 μ L Plasma + 50 μ L 100 mM NaOH + 1.5 mL ethyl acetate, vortex for 15 s, centrifuge at 18000 g for 1 min. Remove 1 mL of the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, dissolve the residue in 250 μ L 70 ng/mL sulfamethoxazole in mobile phase, inject a 10-100 μ L aliquot.

HPLC VARIABLES

Guard column: 40 \times 4.6 5 μ m Nucleosil C18

Column: 120 \times 4.6 5 μ m Nucleosil C18

Mobile phase: MeOH:70 mM pH 4.75 KH_2PO_4 , 25:75

Flow rate: 1.5

Injection volume: 10-100

Detector: E, Metrohm Model 656 electrochemical and Model 641 VA detectors, working and auxiliary electrodes glassy carbon, reference electrode Ag/AgCl, 1200 mV; UV 280

CHROMATOGRAM

Retention time: 6

Internal standard: sulfamethoxazole (7.5)

Limit of detection: 10 ng/mL (E); 100 ng/mL (UV)

KEY WORDS

plasma; pig; human

REFERENCE

Nordholm, L.; Dalgaard, L. Assay of trimethoprim in plasma and urine by high-performance liquid chromatography using electrochemical detection. *J.Chromatogr.*, **1982**, 233, 427-431

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μ L Serum, plasma, or CSF + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 \times 2.1 40 μ m preparative grade C18 (Analytichem); B 250 \times 4.6 10 μ m 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: 6.75
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

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

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, CSF, gastric fluid, urine
Sample preparation: 200 μ L Serum, urine, CSF, or gastric fluid + 300 μ L reagent. Flush column A to waste with 500 μ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μ L 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 hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μ m preparative grade C18 (Analytichem); B 75 \times 2.1 pellicular C18 (Whatman) + 250 \times 4.6 5 μ m 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: 6.5
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiasepoxide, 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

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, peritoneal fluid, synovial fluid, tissue, urine

Sample preparation: Condition a C18 SPE cartridge (J.T. Baker) with 1 mL MeOH, 1 mL water, and 1 mL 100 mM pH 4.5 acetate buffer. CSF, peritoneal fluid, serum, synovial fluid, tissue. Homogenize (TenBroeck tissue grinder) endometrial tissue with saline, centrifuge at 510 g, remove supernatant. Centrifuge fluids at 510 g. 1 mL Sample + 1 mL 100 mM acetate buffer, mix, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeCN:MeOH:100 mM acetate buffer 12.5:12.5:75, add 12.5 ng ormetoprim, inject an aliquot. Urine. 1 mL Urine + 1 mL MeCN, centrifuge at 510 g for 10 min. Remove 25 μ L of the supernatant and add it to 1 mL 100 mM acetate buffer, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeCN:MeOH:100 mM acetate buffer 12.5:12.5:75, add 12.5 ng ormetoprim, inject an aliquot.

HPLC VARIABLES

Column: C18 (Rainin)

Mobile phase: MeCN:MeOH:50 mM phosphate buffer 12.5:12.5:75, pH 3.0

Flow rate: 1.5

Detector: UV 280

CHROMATOGRAM

Retention time: 6

Internal standard: ormetoprim (8)

Limit of detection: 15 ng/mL

OTHER SUBSTANCES

Extracted: sulfamethoxazole

KEY WORDS

serum; endometrium; SPE; horse; pharmacokinetics

REFERENCE

Brown, M.P.; Gronwall, R.; Castro, L. Pharmacokinetics and body fluid and endometrial concentrations of trimethoprim-sulfamethoxazole in mares. *Am.J.Vet.Res.*, **1988**, *49*, 918–922

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: 50 μ L Serum, urine, or CSF + 50 μ L 100 μ g/mL antipyrine in MeOH, vortex for 15 s, centrifuge at 10000 g for 2 min, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 37-50 μ m μ Bondapak C18

Column: 300 \times 3.9 10 μ m μ Bondapak C18

Mobile phase: MeOH:buffer 35:65 (Buffer was 97% 67 mM KH_2PO_4 + 3% 67 mM Na_2HPO_4 and the pH was adjusted to 3.5 with phosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 4.6

Internal standard: antipyrine (11.0)

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: N-acetylsulfamethoxazole, sulfamethoxazole

KEY WORDS

serum

REFERENCE

Weber, A.; Opheim, K.E.; Siber, G.R.; Ericson, J.F.; Smith, A.L. High-performance liquid chromatography quantitation of trimethoprim, sulfamethoxazole, and N4-acetylsulfamethoxazole in body fluids. *J.Chromatogr.*, **1983**, *278*, 337-345

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 500 μ L Plasma + 100 μ L 30 μ g/mL sulfamethazine (sulfadimidine) in EtOH + 150 μ L 3% trichloroacetic acid in EtOH + 100 μ L EtOH, vortex, freeze at -20° for 5 min, centrifuge, freeze at -20° for 10 min, centrifuge through a Spin-X filter tube, inject a 10 μ L aliquot of the supernatant. Tissue. 1-3 g Tissue + 3 (muscle) or 6 (liver) μ L 1 mg/mL sulfamethoxazole in EtOH + 2 (liver) or 3 (muscle) mL 0.7% trichloroacetic acid in acetone, mix in Whirlmixer, sonicate for 10 min at 40° , add 2 mL 10 mM pH 6 Na_2HPO_4 , sonicate for 5 min, add 100 μ L 500 mM NaOH, add 9 (muscle) or 10 (liver) mL dichloromethane, mix thoroughly for 1 min, centrifuge at 2240 g for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness at 40° under a stream of nitrogen. Dissolve the residue in 400 (muscle) or 800 (liver) μ L MeCN:10 mM pH 2.8 phosphate buffer 20:80, sonicate, wash with 1 mL hexane. Sonicate the aqueous phase for 1 min, centrifuge through a Spin-X filter tube, inject a 10 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20×4.6 5 μ m Supelcosil LC-18 DB

Column: 250×4.6 5 μ m Supelcosil LC-18 DB

Mobile phase: MeCN:buffer 23:77 (plasma) or 20:80 (tissue) with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)

Flow rate: 0.9

Injection volume: 10

Detector: UV 270

CHROMATOGRAM

Retention time: 9 (plasma), 14 (tissue)

Internal standard: sulfamethazine (sulfadimidine) (8), sulfamethoxazole (18)

Limit of quantitation: 80 ng/g (muscle); 250 mg/mL (plasma)

OTHER SUBSTANCES

Simultaneous: sulfadiazine

KEY WORDS

plasma; fish; salmon; trout; muscle; liver

REFERENCE

Hormazabal, V.; Rogstad, A. Simultaneous determination of sulphadiazine and trimethoprim in plasma and tissues of cultured fish for residual and pharmacokinetic studies. *J.Chromatogr.*, **1992**, *583*, 201-207

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. 320 μ L Whole blood + 450 μ L urea (1:1) + 30 μ L 2 μ g/mL sulfamethazine, shake mechanically for 15 min, filter (Amicon Micropartition System MPS-1) while centrifuging at 4° at 4000 rpm for at least 3 h, inject a 300 μ L aliquot of the ultrafiltrate. Urine. Dilute urine if necessary. 320 μ L Urine + 30 μ L 2 μ g/mL sulfamethazine, shake mechanically for 15 min, filter (Amicon Micropartition System MPS-1) while centrifuging at 4° at 4000 rpm for at least 3 h, inject a 300 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 150 mm long 5 μ m Spherisorb ODS 2

Mobile phase: MeOH:MeCN:10 mM pH 5.0 sodium acetate buffer containing 4 mM triethylamine 14:14:72

Flow rate: 0.8

Injection volume: 300

Detector: UV 275

CHROMATOGRAM

Internal standard: sulfamethazine

Limit of quantitation: 200 nM

KEY WORDS

fish; whole blood; trout; rainbow trout; pharmacokinetics

REFERENCE

Tan, W.P.; Wall, R.A. Disposition kinetics of trimethoprim in rainbow trout (*Oncorhynchus mykiss*). *Xenobiotica*, 1995, 25, 315–329

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute 1 mL urine with 5 or 10 mL water. 1 mL Serum, plasma, or diluted urine + 200 μ L 1 M pH 6.8 KH_2PO_4 buffer + 6 mL ethyl acetate, vortex for 3 min, centrifuge at 2400 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μ L 30 μ g/mL sulfadimethoxine in mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 25-40 μ m LiChroprep Si 60 (Merck)

Column: 250 \times 4 10 μ m LiChrosorb Si 60

Mobile phase: Dichloromethane:MeOH: ammonia 80:19:1

Flow rate: 1.5

Injection volume: 20

Detector: UV 289

CHROMATOGRAM

Retention time: 2.6

Internal standard: sulfadimethoxine (3.7)

Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Extracted: N-acetylsulfadiazine, sulfadiazine

KEY WORDS

normal phase; serum; plasma; pharmacokinetics

REFERENCE

Ascalone, V. Assay of trimethoprim, sulfadiazine and its N4-acetyl metabolite in biological fluids by normal-phase high-performance liquid chromatography. *J.Chromatogr.*, **1981**, *224*, 59-66

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 sulfadiazine 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: 3

Internal standard: sulfadiazine (6)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: p-aminobenzoic acid, dibromopropamidine isethionate, sulfamerazine

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: 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 K₂HPO₄

Flow rate: 2

Detector: UV 280

CHROMATOGRAM

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Also analyzed: brodimoprim

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 sulfonamides and trimethoprim. *J.Antimicrob.Chemother.*, **1986**, *17*, 489-498

SAMPLE**Matrix:** feed**Sample preparation:** Weigh out 1 g ground feed, add 1 mL 1 mg/mL sulfamethazine in water, add 3 mL trichloroacetic acid solution, mix well, sonicate at 40° for 10 min, make up to 500 mL with MeCN:10 mM Na₂HPO₄ adjusted to pH 3 with phosphoric acid 20:80, mix well, filter a 500 µL aliquot (Costar spin-X (low type) 0.22 µm cellulose acetate) with centrifuging for 1 min, inject a 10 µL aliquot of the filtrate. (Prepare trichloroacetic acid solution by mixing 87 g trichloroacetic acid with 13 g water, add 0.7 mL of this solution to 99.3 mL acetone.)

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 containing 0.1% triethylamine:10 mM pH 2.8 Na₂HPO₄ 21:79**Flow rate:** 0.9**Injection volume:** 10**Detector:** UV 270

CHROMATOGRAM**Retention time:** 7**Internal standard:** sulfamethazine (sulfadimidine) (8)**Limit of quantitation:** 250 µg/g (muscle); 250 mg/mL (plasma)

OTHER SUBSTANCES**Extracted:** sulfadiazine

REFERENCE

Hormazabal, V.; Steffanak, I.; Yndestad, M. Simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by high-performance liquid chromatography. *J. Chromatogr.*, 1993, 648, 183–186

SAMPLE**Matrix:** formulations**Sample preparation:** Powder tablets, add 40 mg trimethoprim, dissolve in 70 mL MeOH, filter (paper), wash filter with MeOH, make up filtrate to 100 mL with MeOH. Dilute a 1 mL aliquot to 10 mL, inject a 10 µL aliquot.

HPLC VARIABLES**Column:** 250 × 4 10 µm Nucleosil C18**Mobile phase:** MeOH:MeCN:water:triethylamine 20:20:60:0.15, pH adjusted to 3.0 with phosphoric acid**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV 240

CHROMATOGRAM**Retention time:** 3**Internal standard:** trimethoprim

OTHER SUBSTANCES**Simultaneous:** nalidixic acid, phenazopyridine

KEY WORDS

tablets; trimethoprim is IS

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: formulations

Sample preparation: Tablets. Powder tablets, weigh out an amount equivalent to about 100 mg trimethoprim, suspend in MeOH, sonicate for 2 min, filter, dilute with MeOH to 250 µg/mL. Suspensions. Dilute with MeOH to 250 µg/mL, sonicate, filter.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-3

Mobile phase: MeCN:water 25:75 containing 1% ammonium acetate, pH 6.90 ± 0.1

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: degradation products, methyl p-hydroxybenzoate, sulfamethoxazole

Noninterfering: n-propyl p-hydroxybenzoate

KEY WORDS

tablets; suspensions; stability-indicating

REFERENCE

Bergh, J.J.; Breytenbach, J.C.; Du Preez, J.L. High-performance liquid chromatographic analysis of trimethoprim in the presence of its degradation products. *J.Chromatogr.*, **1990**, *513*, 392–396

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Crush tablets, weigh out amount equivalent to about 40 mg trimethoprim, dissolve in EtOH, filter, make up to 200 mL with EtOH. Suspensions. Dilute 5 mL suspension to 100 mL with EtOH, filter.

HPLC VARIABLES

Column: 250 × 4.6 7 µm Zorbax TMS

Mobile phase: MeCN:1-propanol:MeOH:THF:acetic acid:water 5:20:15:25:1:34

Flow rate: 2

Injection volume: 20

Detector: UV 271

CHROMATOGRAM

Retention time: 4.37

OTHER SUBSTANCES

Simultaneous: degradation products, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sulfamethoxazole

KEY WORDS

tablets; suspensions; stability-indicating

REFERENCE

Bergh, J.J.; Breytenbach, J.C. Stability-indicating high-performance liquid chromatographic analysis of trimethoprim in pharmaceuticals. *J.Chromatogr.*, **1987**, *387*, 528–531

SAMPLE

Matrix: reaction mixtures

Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 4.6 5 μ m Microsorb C8

Column: 250 \times 4.6 5 μ m Microsorb C8

Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 30:70

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 6.7

Limit of detection: 200 ng/mL

REFERENCE

Lunn, G.; Rhodes, S.W.; Sansone, E.B.; Schmuft, 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 \times 0.35 5 μ m Vydac IDI-TP C18 TMS capped

Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)

Column temperature: 30

Flow rate: 0.006

Injection volume: 1

Detector: UV 270

CHROMATOGRAM

Retention time: 27.5

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS

capillary HPLC

REFERENCE

Ricci, M.C.; Cross, R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming. *J.Liq.Chromatogr.& Rel.Technol.*, **1996**, *19*, 547-564

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped

Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)

Column temperature: 30

Flow rate: 0.006

Injection volume: 1

Detector: UV 270

CHROMATOGRAM

Retention time: 31

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS

capillary HPLC

REFERENCE

Ricci, M.C.; Cross, R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions. *J.Liq.Chrom.Rel.Technol.*, **1996**, *19*, 365-381

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: 6.67 (A), 3.65 (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, hydroxyzine, 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, methyl dopa, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluopromazine, trimeprazine, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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 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, buprenorphine, buprenorphine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphe-

nesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarín, danazol, danthron, dapsoné, 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, fenopropfen, 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, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phen-suximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyriline, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, 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 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:buffer 16:84 (Buffer was 0.1% trifluoroacetic acid adjusted to pH 3 with ammonium hydroxide.)

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM**Retention time:** k' 3.0

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:** Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES**Guard column:** Guard-PAK C18 (Waters)**Column:** 150 \times 3.9 5 μm NOVA PAK C18**Mobile phase:** MeOH:50 mM pH 6.0 KH_2PO_4 35:65**Flow rate:** 0.6**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5.8

REFERENCE

Koga, H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes. *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 10 $\mu\text{g}/\text{mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 125 \times 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:** 1.94

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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,

fantanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, mepytazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phenathienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phenotolamine, 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, tranlycypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimipramine, tripeleppamine, 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: urine

Sample preparation: Condition a 1 mL 100 mg Bond Elut CN SPE cartridge with 3 mL MeCN and 3 mL water. 1 mL Urine + 1.1 mL water, add to the SPE cartridge, wash with 3 mL water, elute with 1 mL MeCN:water 15:85 containing 0.75% triethylamine and 0.375% phosphoric acid (85%), inject a 200 μ L aliquot.

HPLC VARIABLES

Guard column: 15 \times 3.2 7 μ m Aquapore C18 (Brownlee)

Column: 100 \times 4.6 5 μ m Hypersil ODS C18

Mobile phase: Gradient. MeCN:buffer from 9:91 to 35:65, re-equilibrate at initial conditions for 5 min. (Buffer was 0.16% triethylamine containing 0.08% orthophosphoric acid (85%), pH 4.2.)

Flow rate: 1.5

Injection volume: 200

Detector: UV 241

CHROMATOGRAM

Retention time: 5.8

Internal standard: trimethoprim

OTHER SUBSTANCES

Extracted: trimetrexate

KEY WORDS

SPE; trimethoprim is IS

REFERENCE

Tinsley, P.W.; LaCreta, F.P. Improved chromatographic method for the determination of trimetrexate in urine. *J.Chromatogr.*, **1990**, *529*, 468–472

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- Mokry, M.; Klimes, J.; Zahradnicek, M. HPLC analysis of some sulfonamides in selected pharmaceutical formulations. *Pharmazie*, **1994**, *49*, 333–335 [tablets; injections; acetanilide (IS); simultaneous sulfamethoxazole; also phenacetin, phenazone, phenobarbital, phthalylsulfathiazole, sulfadimidine, sulfamethoxydiazine, sulfamoxole, sulfisoxazole]
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- Endoh, Y.S.; Takahashi, Y.; Nishikawa, M. HPLC determination of sulfonamides, their N4-acetyl metabolites and diaminopyrimidine coocidiostats in chicken tissues. *J.Liq.Chromatogr.*, **1992**, *15*, 2091–2110 [skin; plasma; muscle; liver; kidney; LOD 20-50 ng/g; also N-acetyldiaveridine, N-acetylsulfadiazine, N-acetylsulfadimethoxine, N-acetylsulfamethoxazole, N-acetylsulfamonomethoxine, N-acetylsulfaquinolaxine, diaveridine, ormethoprim, sulfadiazine, sulfadimethoxine, sulfamethoxazole, sulfamonomethoxine, sulfaquinolaxine]
- Van't Klooster, G.A.E.; Kolker, H.J.; Woutersen-Van Nijnanten, F.M.A.; Noordhoek, J.; Van Miert, A.S.J.P.A.M. Determination of trimethoprim and its oxidative metabolites in cell culture media and microsomal incubation mixtures by high-performance liquid chromatography. *J.Chromatogr.*, **1992**, *579*, 355–360 [rat; liver; diaveridine (IS); LOD 150 ng/mL]
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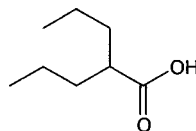
- Tu, Y.H.; Wang, D.-P.; Allen, L.V., Jr. Stability of a nonaqueous trimethoprim preparation. *Am.J.Hosp.Pharm.*, **1989**, *46*, 301–304 [propylene glycol; dimethylacetamide; LOQ 1 µg/mL]
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Valproic Acid

Molecular formula: C₈H₁₆O₂

Molecular weight: 144.2

CAS Registry No.: 99-66-1, 1069-66-5 (sodium salt), 76584-70-8 (semisodium salt)



SAMPLE

Matrix: blood

Sample preparation: Prepare ultrafiltrate from serum with an Amicon Centrifree unit by centrifuging at 700 g for 10 min. 25 μ L Ultrafiltrate + 475 μ L 10 μ g/mL undecylenic acid in MeCN, centrifuge. Remove 50 μ L supernatant, add 100 μ L 18-crown-6 solution, add 50 μ L 1 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, let stand in the dark at 65° for 30 min, inject a 5 μ L aliquot. (Prepare 18-crown-6 solution by dissolving 100 mg potassium carbonate in 50 μ L water, add 5 mL 20 mM 18-crown-6 in MeCN, sonicate for 30 min, add 5 mL MeCN.)

HPLC VARIABLES

Column: 100 \times 2.1 5 μ m HP Hypersil-ODS

Mobile phase: MeOH:water 80:20

Column temperature: 40

Flow rate: 0.3

Injection volume: 5

Detector: F ex 322 em 695

CHROMATOGRAM

Retention time: 2.5

Internal standard: undecylenic acid (4.5)

Limit of quantitation: 6.25 μ g/mL

OTHER SUBSTANCES

Noninterfering: carbamazepine, phenobarbital, phenytoin

KEY WORDS

serum; derivatization

REFERENCE

Liu, H.; Forman, L.J.; Montoya, J.; Eggers, C.; Barham, C.; Delgado, M. Determination of valproic acid by high-performance liquid chromatography with photodiode-array and fluorescence detection. *J.Chromatogr.*, **1992**, 576, 163–169

SAMPLE

Matrix: blood

Sample preparation: Filter (Amicon Centrifree) serum at 550 g for 15 min. Remove a 25 μ L aliquot of the ultrafiltrate and add it to 475 μ L 1 μ g/mL undecylenic acid in MeCN, centrifuge. Remove a 50 μ L aliquot of the supernatant and add it to 100 μ L 18-crown-6 suspension and 50 μ L 1 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, heat at 65° in the dark for 30 min, inject a 5 μ L aliquot. (Prepare the 18-crown-6 suspension by dissolving 100 mg potassium carbonate in 50 μ L water then adding this mixture to 5 mL 20 mM 18-crown-6 in MeCN, sonicate for 30 min, add 5 mL MeCN.)

HPLC VARIABLES

Column: 100 \times 2.1 5 μ m HP Hypersil-ODS

Mobile phase: MeOH:water 80:20

Column temperature: 40

Flow rate: 0.3

Injection volume: 5**Detector:** UV 200; UV 320; F ex 322 em 695

CHROMATOGRAM**Retention time:** 2.5**Internal standard:** undecylenic acid (4.4)**Limit of detection:** 1.25 ng/mL (S/N 12, fluorescence)

OTHER SUBSTANCES

Noninterfering: acetaminophen, carbamazepine, chlordiazepoxide, clonazepam, desmethyldiazepam, diazepam, digoxin, disopyramide, ethosuximide, gentamicin, lidocaine, lorazepam, methotrexate, nitrazepam, oxazepam, phenobarbital, phenytoin, prazepam, primidone, procainamide, quinidine, temazepam, theophylline, vancomycin

KEY WORDS

serum; derivatization

REFERENCE

Liu, H.; Montoya, J.L.; Forman, L.J.; Eggers, C.M.; Barham, C.F.; Delgado, M. Determination of free valproic acid: evaluation of the Centrifree system and comparison between high-performance liquid chromatography and enzyme immunoassay. *Ther.Drug Monit.*, **1992**, *14*, 513–521

SAMPLE**Matrix:** blood

Sample preparation: 200 μ L Plasma + 500 μ L water, shake, add 300 μ L 25 mM perchloric acid, add 3 mL cyclohexane, vortex for 3 min, centrifuge at 3500 rpm for 5 min, repeat extraction twice more. Combine the organic layers and dry them over anhydrous sodium sulfate, add 1 μ mole sodium methoxide in MeOH, shake for 1 min, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute with 200 μ L MeCN, add 100 μ L buffer, add 200 μ L reagent, heat at 70° for 40 min, inject an aliquot. (Prepare the buffer by dissolving 3.8 g KH_2PO_4 and 5.96 g Na_2HPO_4 in 200 mL water, pH 7.4. The reagent was 17 mg/mL 2-naphthacyl bromide in MeCN containing 1 mg/mL dicyclohexane-18-crown-6.)

HPLC VARIABLES**Column:** 125 mm long 3 μ m HS C18 (Perkin-Elmer)**Mobile phase:** MeOH:water 77:23**Flow rate:** 1**Injection volume:** 6**Detector:** UV 280

CHROMATOGRAM**Retention time:** 5.5**Limit of detection:** 3.47 μ g/mL

KEY WORDS

plasma; derivatization; comparison with TLC

REFERENCE

Corti, P.; Cenni, A.; Corbini, G.; Dreassi, E.; Murratzu, C.; Caricchia, A.M. Thin-layer chromatography and densitometry in drug assay: comparison of methods for monitoring valproic acid in plasma. *J.Pharm.Biomed.Anal.*, **1990**, *8*, 431–436

SAMPLE**Matrix:** blood

Sample preparation: Perform all operations with the exclusion of light. Evaporate 240 μ L derivatization solution into a vial, add 400 μ L 50 mM pH 7.0 phosphate buffer, add 100 μ L plasma, add 10 μ L 46 μ g/mL undecylenic acid in MeCN, vortex for 5 s, heat at 70° for 40 min, add 500 μ L MeCN, centrifuge at 3000 g for 5 min, inject a 20 μ L aliquot. (Derivatization solution was 1.65 g Arkopal N-130 (a non-ionic surfactant, nonylphenol/13 unit chain polyoxyethylene) + 650 mg tetrahexylammonium bromide + 60 mg 4-bromo-methyl-7-methoxycoumarin in 20 mL acetone.)

HPLC VARIABLES

Guard column: 10 \times 3 5-20 μ m LiChroprep RP-8

Column: 100 \times 3 5 μ m Chromspher C18

Mobile phase: Gradient. MeOH:water 80:20 for 3 min, then to 100:0 over 6 min, then held at 100:0 for 4 min.

Injection volume: 20

Detector: F ex 330 em 395

CHROMATOGRAM

Retention time: 4

Internal standard: undecylenic acid (7)

Limit of detection: 1 μ g/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: carbamazepine, phenobarbital, phenytoin

KEY WORDS

plasma; derivatization

REFERENCE

van der Horst, F.A.; Eikelboom, G.G.; Holthuis, J.J. High-performance liquid chromatographic determination of valproic acid in plasma using a micelle-mediated pre-column derivatization. *J.Chromatogr.*, **1988**, *456*, 191-199

SAMPLE

Matrix: blood

Sample preparation: 250 μ L Serum + 500 μ L 250 ng/mL diazepam in MeCN, vortex for 15 s, centrifuge at 1400 g for 10 min, inject a 25 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Zorbax ODS

Mobile phase: Gradient. MeCN:buffer 37:63 for 9 min, to 60:40, maintain at 60:40 for 1.5 min, re-equilibrate at initial conditions for 3 min. (Buffer was 10 mM NaH₂PO₄ adjusted to pH 2.3 with phosphoric acid.)

Column temperature: 40

Flow rate: 2.5

Injection volume: 25

Detector: UV 210

CHROMATOGRAM

Retention time: 5.1

Internal standard: diazepam (7.7)

Limit of detection: 1.5 μ g/mL

OTHER SUBSTANCES

Noninterfering: carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone

KEY WORDS

serum; pharmacokinetics

REFERENCE

Lovett, L.J.; Nygard, G.A.; Erdmann, G.R.; Burley, C.Z.; Wahba Kahlil, S.K. HPLC determination of valproic acid in human serum using ultraviolet detection. *J.Liq.Chromatogr.*, **1987**, *10*, 687-699

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Plasma + 200 μ L 1 M HCl saturated with ammonium sulfate, vortex for 20 s, add 60 μ L 10 μ g/mL 4-methylprimidone in MeCN, vortex for 20 s, centrifuge at 2700 g for 5 min, inject a 5-10 μ L aliquot of the MeCN layer.

HPLC VARIABLES

Column: 250 \times 4.5 μ m LiChrosorb RP-18

Mobile phase: MeOH:THF:50 mM pH 5.9 phosphate buffer 44:1:55

Column temperature: 50

Flow rate: 1.1

Injection volume: 5-10

Detector: UV 210

CHROMATOGRAM

Retention time: 6.5

Internal standard: 4-methylprimidone (5)

Limit of detection: 2.6 μ g/mL

OTHER SUBSTANCES

Extracted: carbamazepine, phenobarbital, phenytoin, primidone

Simultaneous: acetaminophen, caffeine, chloramphenicol, diazepam, ethosuximide, ethylphenylmalonamide, glutethimide, lidocaine, methylphenobarbital, pentobarbital, salicylic acid, theophylline

KEY WORDS

plasma

REFERENCE

Kushida, K.; Ishizaki, T. Concurrent determination of valproic acid with other antiepileptic drugs by high-performance liquid chromatography. *J.Chromatogr.*, **1985**, *338*, 131-139

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 100 μ L 500 μ g/mL hexanoic acid + 500 μ L pH 2.2 phosphate buffer + 5 mL hexane, shake for 5 min, centrifuge for 1 min. Remove 4 mL of the organic layer and add it to 50 μ L 200 mM NaOH, extract for 2 min, centrifuge for 1 min, inject a 20 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 100 \times 3 octyl CP-tm-Spher C8 glass column (Chrompack)

Mobile phase: MeCN:500 mM NaH₂PO₄ 35:65 adjusted to pH 2.2 with phosphoric acid

Flow rate: 0.9

Injection volume: 20

Detector: UV 210

CHROMATOGRAM

Retention time: 7.3

Internal standard: hexanoic acid (3)

Limit of detection: 5 $\mu\text{g/mL}$

OTHER SUBSTANCES

Simultaneous: metabolites

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: 50 μL Serum + 1 mL 4 $\mu\text{g/mL}$ IS in MeCN, vortex for 10 s, centrifuge. Remove an 800 μL aliquot of the supernatant and add it to 200 μL 3 mg/mL α -bromoacetophenone in MeCN and 100 μL triethylamine, heat in an open tube at 80° for 30 min, cool, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Finepak Sil C_{18.5} (Japan Spectroscopic, Tokyo)

Mobile phase: MeCN: water 60:40

Column temperature: 30

Flow rate: 1

Injection volume: 10

Detector: UV 245

CHROMATOGRAM

Retention time: 21

Internal standard: cyclohexane carboxylic acid (13)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: hexobarbital, phenobarbital

Noninterfering: carbamazepine, phenytoin

KEY WORDS

serum; derivatization

REFERENCE

Nakamura, M.; Kondo, K.; Nishioka, R.; Kawai, S. Improved procedure for the high-performance liquid chromatographic determination of valproic acid in serum as its phenacyl ester. *J.Chromatogr.*, **1984**, *310*, 450-454

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 25 μL buffer + 250 μL 167 $\mu\text{g/mL}$ nonanoic acid in MeCN, vortex for 10 s, centrifuge for 5 min. Remove a 200 μL aliquot of the supernatant and add it to 50 μL 20 mg/mL 4-bromophenacyl bromide in MeCN containing 500 $\mu\text{g/mL}$ dicyclohexane-18-crown-6, heat at 70° for 15 min, cool, inject a 5 μL aliquot. (Prepare buffer by dissolving 19.04 g KH_2PO_4 and 37.4 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 1 L water, pH 7.0.)

HPLC VARIABLES

Column: 100 \times 5 5 μm Hypersil ODS

Mobile phase: MeCN:3 mM KH₂PO₄ 70:30
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: nonanoic acid (6)
Limit of detection: 60 μM

OTHER SUBSTANCES

Noninterfering: acetaminophen, clonazepam, diazepam, phenobarbital, phenytoin, primidone, salicylic acid, theophylline

KEY WORDS

horse; serum; derivatization

REFERENCE

Moody, J.P.; Allan, S.M. Measurement of valproic acid in serum as the 4-bromophenacyl ester by high performance liquid chromatography. *Clin.Chim.Acta*, **1983**, *127*, 263–269

SAMPLE

Matrix: blood

Sample preparation: 250 μL Serum + 100 μL 2.5 mM heptanoic acid + 300 μL 2 M sulfuric acid + 1 mL petroleum ether (40-60°), shake for 5 min, centrifuge. Remove a 900 μL aliquot of the organic layer and add it to 10 μL tributylamine, evaporate to dryness at 70° in about 10 min, reconstitute the residue in 400 μL MeCN, add 50 μL 10 mg/mL p-bromophenacyl bromide in MeOH, heat at 70° for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Lichrosorb RP-2
Mobile phase: MeOH:50 mM pH 2.3-2.5 phosphate buffer 75:25
Column temperature: 50
Flow rate: 2
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 3.14
Internal standard: heptanoic acid (2.74)

OTHER SUBSTANCES

Noninterfering: carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone

KEY WORDS

serum; derivatization

REFERENCE

Ehrenthal, W.; Rochel, M. Kontrolle der Valproinsäure-Therapie durch Serumspiegelbestimmungen mit HPLC oder EMIT und durch gleichzeitige Bestimmung klinisch-chemischer Parameter [Drug monitoring for valproic acid with HPLC or EMIT and concomitant measurements of clinical-chemical parameters]. *Arzneimittelforschung*, **1982**, *32*, 449–452

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 50 μ g IS + 100 μ L concentrated phosphoric acid + 2 mL dichloromethane, shake for 30 min, centrifuge at 2500 rpm for 10 min. Remove a 500 μ L aliquot of the organic layer and add it to 100 μ L reagent and 0.5 mg sodium bicarbonate, heat at 75° for 30 min, evaporate to 0.5 mL under a stream of nitrogen, make up to 1 mL with MeCN, inject an aliquot. (Reagent was 20 mM p-bromophenacyl bromide in MeCN containing 1 mM 18-crown-6, prepared by diluting Phenacyl-8 (Pierce) 5 times with MeCN.)

HPLC VARIABLES

Column: C18

Mobile phase: MeCN:water 65:35

Detector: UV 254

CHROMATOGRAM

Retention time: 10

Internal standard: cyclohexane carboxylic acid (6.5)

Limit of quantitation: 5 μ g/mL

KEY WORDS

serum; derivatization

REFERENCE

Kline, W.F.; Enagonio, D.P.; Reeder, D.J.; May, W.E. Liquid chromatographic determination of valproic acid in human serum. *J.Liq.Chromatogr.*, **1982**, *5*, 1697–1709

SAMPLE

Matrix: blood, dialysate

Sample preparation: Blood. 20 μ L Plasma or whole blood + 1 mL 1 μ g/mL nonanoic acid in MeCN, centrifuge. Remove 100 μ L supernatant, add 100 μ L suspension, 15 min before injection add 200 μ L 0.5 mg/mL bromomethylmethoxycoumarin, mix, inject. Dialysate. Lyophilize, add 40 μ L suspension (prepared with twice the amount of crown ether), add 40 μ L 0.5 mg/mL bromomethylmethoxycoumarin, mix, inject after 15 min. (Suspension was 100 mg potassium carbonate + 50 μ L water + 5 mL 20 mM 18-crown-6 in MeCN, sonicate for 1 h, add 5 mL MeCN, separate the suspension from the precipitated potassium carbonate.)

HPLC VARIABLES

Column: 200 \times 3 μ m Chromspher ODS

Mobile phase: MeCN:2.5 M formic acid 75:25

Flow rate: 0.4

Detector: F ex 325 em 398 (cut-off filter)

CHROMATOGRAM

Retention time: 8, 11 (double peak caused by impurity in derivatizing reagent)

Internal standard: nonanoic acid (12, 16)

Limit of quantitation: 1 μ g/mL

KEY WORDS

plasma; whole blood; brain; derivatization; human; rat

REFERENCE

Wolf, J.H.; Veenma-van der Duin, L.; Korf, J. Automated analysis procedure for valproic acid in blood, serum and brain dialysate by high-performance liquid chromatography with bromomethylmethoxycoumarin as fluorescent label. *J.Chromatogr.*, **1989**, *487*, 496–502

SAMPLE

Matrix: formulations

Sample preparation: Condition a Tech Elut C18 SPE cartridge with 3 mL MeOH and 2 mL water. Weigh out powdered tablets containing 40 mg sodium valproate, add 100 mL water, stir for 10 min, filter (paper), acidify the filtrate with phosphoric acid, add a 2 mL aliquot to the SPE cartridge, wash with two 2 mL portions of water, dry under vacuum, elute with 3 mL MeCN, dilute the eluate to 5 mL with MeCN. Mix 500 μ L of this solution with 300 μ L 178 μ g/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN, add 50 μ L 3% triethylamine in MeCN, heat at 70° for 30 min, cool, add 50 μ L 60.84 μ g/mL IS in MeCN, inject a 50 μ L 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 \times 4.6 5 μ m Hypersil RP-8

Mobile phase: MeCN:water 68:32

Flow rate: 1

Injection volume: 50

Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 7

Internal standard: 6-methoxynaphthacyl ester of nonanoic acid (Dissolve 2 mmole nonanoic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μ L 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 nonanoic acid (mp 66-8°).) (10)

Limit of detection: 2 pmole

OTHER SUBSTANCES

Simultaneous: decanoic acid, dodecanoic acid, heptanoic acid, undecanoic acid

Interfering: octanoic acid

KEY WORDS

derivatization; SPE; tablets

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: formulations

Sample preparation: Weigh out capsule contents equivalent to 250 mg valproic acid, add 80 mL MeOH, sonicate for 10 min, make up to 100 mL with MeOH, filter, allow to settle for 30 min. Dilute a 4 mL aliquot to 100 mL with mobile phase. Mix a 20 mL aliquot of the diluted solution with 10 mL 50 μ g/mL ibuprofen in mobile phase, make up to 100 mL with mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m Spheri-5 RP-8

Mobile phase: MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na₂HPO₄ and 7 mM KH₂PO₄ to achieve pH 7.)

Flow rate: 1

Injection volume: 50

Detector: F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 $\mu\text{g}/\text{mL}$ reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 mm \times 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α -(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamionitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetonitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α -(3,4-dimethoxyphenyl)-4'-methylcinnamionitrile. Dissolve 20 mmoles α -(3,4-dimethoxyphenyl)-4'-methylcinnamionitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h, cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α -(3,4-dimethoxyphenyl)-4'-bromomethylcinnamionitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α -(3,4-dimethoxyphenyl)-4'-bromomethylcinnamionitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α -(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamionitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α -(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamionitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α -(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamionitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM

Retention time: k' 0.3256

Internal standard: ibuprofen (k' 4.124)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: ketoprofen, mefenamic acid, naproxen, probenecid, salicylic acid

KEY WORDS

capsules; post-column extraction

REFERENCE

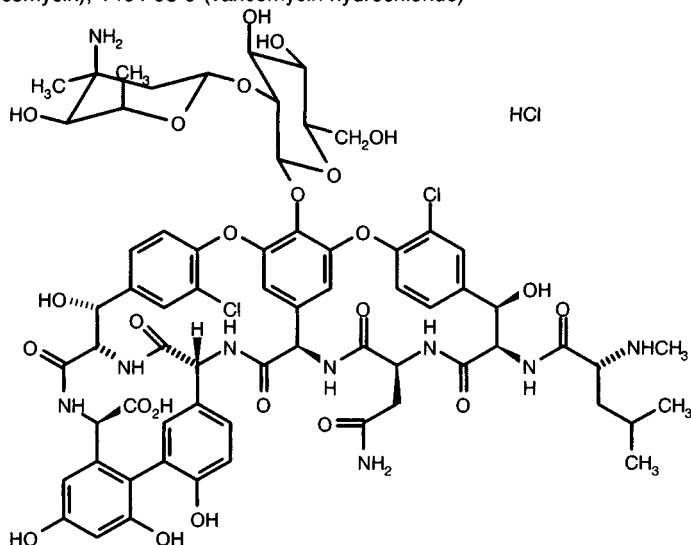
Kim, M.; Stewart, J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α -phenylcinnamionitrile quaternary ammonium salt as a new fluorescent ion-pair reagent. *J.Liq.Chromatogr.*, **1990**, *13*, 213-237

Vancomycin

Molecular formula: $C_{66}H_{75}Cl_2N_9O_{24}$

Molecular weight: 1449.2

CAS Registry No.: 1404-90-6 (vancomycin), 1404-93-9 (vancomycin hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 50 μ L Serum + 9 μ L 125 μ g/mL trimethoprim + 75 μ L acetone: 10% trichloroacetic acid 1:2, vortex for 5 s, centrifuge for 4 min. Remove 62.5 μ L of the supernatant and add it to 62.5 μ L 50 mM KH_2PO_4 , add 250 μ L diethyl ether, vortex for 10 s, centrifuge for 5 min, filter (0.45 μ m) the lower aqueous layer, inject a 20 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 75 \times 4.6 TSK gel ODS-80TM (Tosoh)

Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 , 8:92

Flow rate: 1

Injection volume: 20

Detector: UV 235

CHROMATOGRAM

Retention time: 12

Internal standard: trimethoprim (30)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS

serum; comparison with fluorescence polarization immunoassay

REFERENCE

Morishige, H.; Shuto, H.; Ieiri, I.; Otsubo, K.; Oishi, R. Instability of standard calibrators may be involved in overestimating vancomycin concentrations determined by fluorescence polarization immunoassay. *Ther. Drug Monit.*, **1996**, *18*, 80–85

SAMPLE

Matrix: blood

Sample preparation: 50 μ L Serum or plasma + 25 μ L 25 μ g/mL ristocetin in water + 10 μ L 15% perchloric acid, vortex for 5 min, centrifuge at 8° at 13000 g for 5 min, inject a 50 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: amino (Burdick and Jackson)

Column: 250 \times 4.6 5 μ m Microsorb-MV NH₂ or 250 \times 4.6 5 μ m CA AM5 amino propyl (Burdick and Jackson)

Mobile phase: MeCN:20 mM NaH₂PO₄:20 mM Na₂HPO₄ 62:14.25:23.75

Flow rate: 2

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 5.5

Internal standard: ristocetin (8.9)

Limit of detection: 320 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, amlodipine, carbamazepine, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, cisapride, clindamycin, clonidine, codeine, cyclosporine, digoxin, diphenhydramine, disopyramide, ethosuximide, fluconazole, gentamicin, gentamicin, heparin, labetalol, levothyroxine, lidocaine, lithium, methotrexate, metronidazole, minoxidil, nafcillin, nifedipine, phenobarbital, phenobarbital, phenytoin, phenytoin, primidone, procainamide, propranolol, quinidine, ranitidine, salicylic acid, theophylline, tobramycin, tobramycin, valproic acid, warfarin

KEY WORDS

serum; plasma

REFERENCE

Li, L.; Miles, M.V.; Hall, W.; Carson, S.W. An improved micromethod for vancomycin determination by high-performance liquid chromatography. *Ther. Drug Monit.*, **1995**, *17*, 366–370

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L 60% perchloric acid, mix, centrifuge. Wash the supernatant with 1 mL dichloromethane, inject a 50 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Nucleosil RP-18

Mobile phase: MeCN:5 mM pH 2.8 KH₂PO₄ 10:90

Column temperature: 30

Flow rate: 1

Injection volume: 50

Detector: UV 229

CHROMATOGRAM**Retention time:** 4.8**Limit of detection:** 200 ng/mL**Limit of quantitation:** 1 µg/mL

OTHER SUBSTANCES**Simultaneous:** acetaminophen, aspirin, caffeine

KEY WORDSplasma

REFERENCELuksa, J.; Marusic, A. Rapid high-performance liquid chromatographic determination of vancomycin in human plasma. *J.Chromatogr.B*, **1995**, *667*, 277–281

SAMPLE**Matrix:** blood**Sample preparation:** 100 µL Plasma + 100 µL cold 40% trichloroacetic acid, mix for 2 min, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 1 mL diethyl ether, shake, discard the ether layer. Remove 50 µL of the aqueous layer and add it to 10 µL 10 ppm phenacetin, mix, inject a 20 µL aliquot.

HPLC VARIABLES**Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeCN:MeOH:50 mM Pic-B7 8:17:75**Flow rate:** 1**Injection volume:** 20**Detector:** UV 280

CHROMATOGRAM**Retention time:** 7.2**Internal standard:** phenacetin (10.8)

KEY WORDSplasma; comparison with fluorescence polarization immunoassay

REFERENCENajjar, T.A.; Al-Dhuwailie, A.; Tekle, A. Comparison of high-performance liquid chromatography with fluorescence polarization immunoassay for the analysis of vancomycin in patients with chronic renal failure. *J.Chromatogr.B*, **1995**, *672*, 295–299

SAMPLE**Matrix:** cell suspensions**Sample preparation:** 100 µL Cell suspension + 100 µL cefazolin 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 KH₂PO₄ 10:90**Flow rate:** 1**Injection volume:** 75**Detector:** UV 254

CHROMATOGRAM**Retention time:** 14**Internal standard:** cefazolin**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

HPLC VARIABLES**Column:** 250 × 4.6 5 μm Bakerbond phenylethyl**Mobile phase:** MeCN:THF:buffer 7:1:92 (Buffer was 4 mL triethylamine in 2 L water, pH adjusted to 3.2 with phosphoric acid.)**Flow rate:** 1**Detector:** UV 280

CHROMATOGRAM**Retention time:** 3.03

KEY WORDS

injections; saline; water; stability-indicating

REFERENCE

Stiles, M.L.; Allen, L.V., Jr.; Prince, S.J. Stability of various antibiotics kept in an insulated pouch during administration via portable infusion pump. *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 70–74

SAMPLE**Matrix:** formulations**Sample preparation:** Dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 300 × 4.6 5 μm Hypersil ODS**Mobile phase:** MeCN:THF:buffer 8.3:1.4:90 (Buffer was 20 mL 1 M triethylamine in 900 mL water, adjusted to pH 3.2 with 1 M NaOH.)**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 216

CHROMATOGRAM**Retention time:** 6.4

OTHER SUBSTANCES**Simultaneous:** aztreonam

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:** formulations**Sample preparation:** Inject a 50 μL aliquot of the dialysis fluid.

HPLC VARIABLES**Column:** 250 \times 3.6 5 μm Spherisorb ODS2**Mobile phase:** MeCN:200 mM ammonium acetate:water 9:10:81 adjusted to pH 5.4 with glacial acetic acid**Flow rate:** 1.5**Injection volume:** 50**Detector:** UV 214

CHROMATOGRAM**Retention time:** 9

KEY WORDS

dialysis solutions; stability-indicating

REFERENCEMawhinney, W.M.; Adair, C.G.; Gorman, S.P.; McClurg, B. Stability of vancomycin hydrochloride in peritoneal dialysis solution. *Am.J.Hosp.Pharm.*, **1992**, *49*, 137-139

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 6-10 μL aliquot.

HPLC VARIABLES**Guard column:** 20 \times 4.6 Supelguard LC-1 (Supelco)**Column:** 250 \times 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:** 1.83**Internal standard:** 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES**Simultaneous:** acetaminophen, acetanilide, N-acetylcysteine, N-acetylprocainamide, amobarbital, aspirin, barbital, butabarbital, butalbital, caffeine, carbamazepine, cimetidine, codeine, cyheptamide, diazoxide, difunisal, disopyramide, ethchlorvynol, ethosuximide, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, mephobarbital, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, pentobarbital, phenacetin, phenobarbital, phensuximide, phenylbutazone, phenytoin, primidone, procainamide, salicylamide, secobarbital, sulindac, thiopental, tolmetin, trimethoprim**Noninterfering:** amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid**Interfering:** ampicillin, chloramphenicol, chlorpropamide, diphylline, salicylic acid, sulfamethoxazole, theophylline

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: solutions

Sample preparation: Inject a 25 μ L aliquot.

HPLC VARIABLES

Guard column: RCSS Guard-Pak (Waters)

Column: 100 \times 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: 1.1

OTHER SUBSTANCES

Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cefamandole, cefazolin, cefotaxime, cefoxitin, cephalixin, cephalothin, cephapirin, chloramphenicol, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin

REFERENCE

Danzer, L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum. *Clin. Chem.*, **1983**, *29*, 856–858

ANNOTATED BIBLIOGRAPHY

Demotes-Mainard, F.; Labat, L.; Vinçon, G.; Bannwarth, B. Column-switching high-performance liquid chromatographic determination of vancomycin in serum. *Ther. Drug Monit.*, **1994**, *16*, 293–297 [cefaloridine (IS); LOD 1 μ g/mL; non-interfering cefoperazone, cefotiam; simultaneous cefonicid, ceforanide, cefotaxime, cefotetan, cefpiramide, cefsulodin, ceftazidime, ceftriaxone, desacetylcefotaxime]

Jenke, D.R. Drug binding by reservoirs in elastomeric infusion devices. *Pharm. Res.*, **1994**, *11*, 984–989 [injections; saline; 5% dextrose]

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Inman, E.L.; Clemens, R.L.; Olsen, B.A. Determination of EDTA in vancomycin by liquid chromatography with absorbance ratioing for peak identification. *J. Pharm. Biomed. Anal.*, **1990**, *8*, 513–520

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Greene, S.V.; Abdalla, T.; Morgan, S.L.; Bryan, C.S. High-performance liquid chromatographic analysis of vancomycin in plasma, bone, atrial appendage tissue and pericardial fluid. *J. Chromatogr.*, **1987**, *417*, 121–128

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Jehl, F.; Gallion, C.; Thierry, R.C.; Monteil, H. Determination of vancomycin in human serum by high-pressure liquid chromatography. *Antimicrob. Agents Chemother.*, **1985**, *27*, 503–507

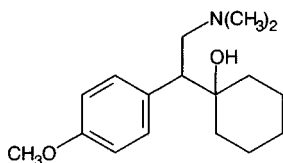
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Venlafaxine

Molecular formula: C₁₇H₂₇NO₂

Molecular weight: 277.4

CAS Registry No.: 93413-69-5 (venlafaxine),
99300-78-4 (venlafaxine hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L 22.5 μ g/mL IS in water + 200 μ L saturated sodium borate, vortex, add 5 mL isopropyl ether (Caution! Isopropyl ether readily forms explosive peroxides!), shake for 15 min, centrifuge at 2500 rpm for 10 min. Remove a 4.5 mL aliquot of the organic phase and add it to 400 μ L 10 mM HCl, shake for 15 min, centrifuge at 2500 rpm for 10 min. Discard the organic phase and add 1 mL saturated sodium borate solution to the aqueous layer, vortex, add 2 mL isopropyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, add 5-10 mg anhydrous sodium carbonate powder to the residue, add 200 μ L 150 μ g/mL naproxen chloride in dichloromethane, vortex, let stand in the dark for 20 h, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL water, vortex, add 2 mL chloroform, shake for 10 min, centrifuge at 2500 rpm for 5 min. Remove the chloroform layer and add it to 2 mL water, shake for 10 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μ L initial mobile phase, inject an 11 μ L aliquot. (Prepare naproxen chloride as follows. React 8.1 g naproxen in 40 mL dichloromethane with 25 g oxalyl chloride at room temperature for 22 h, evaporate to dryness under reduced pressure at 42°, reconstitute the residue with 35 mL dichloromethane, slowly add 1.12 L n-hexane, chill to precipitate crystals, decant solvent carefully and rapidly, dry crystals rapidly under nitrogen, dry crystals of naproxen chloride (mp 95-7°) over calcium chloride in a vacuum desiccator.)

HPLC VARIABLES

Guard column: 20 \times 4.6 Supelguard LC-8-DB (Supelco)

Column: 150 \times 4.6 5 μ m Supelcosil LC-8-DB

Mobile phase: Gradient. MeCN:buffer from 50:50 to 54:46 over 30 min, to 55:45 over 6 min, to 57:43 over 14 min, maintain at 57:43 for 3 min, return to initial conditions over 3 min. (After 30 min increase flow rate to 2 mL/min over 6 min, maintain at 2 mL/min for 17 min, return to initial conditions over 3 min. Buffer was 100 mM KH₂PO₄ adjusted to pH 3.0 with 85% orthophosphoric acid, add 0.07% triethylamine to achieve a final pH of 3.25.)

Flow rate: 0.8

Injection volume: 11

Detector: UV 229

CHROMATOGRAM

Retention time: 16.53 (R), 18.03 (S)

Internal standard: 1-[2-(dimethylamino)-1-(2-chlorophenyl)ethyl]cyclohexanol (Wy-45,818)
(19.74, 22.38 (enantiomers))

Limit of quantitation: 50 ng/mL; 25 ng/mL (22 μ L inj)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

chiral; dog; rat; human; plasma; derivatization; pharmacokinetics

REFERENCE

Wang, C.P.; Howell, S.R.; Scatina, J.; Sisenwine, S.F. The disposition of venlafaxine enantiomers in dogs, rats, and humans receiving venlafaxine. *Chirality*, **1992**, *4*, 84–90

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μ L 15 μ g/mL IS in water + 200 μ L saturated sodium borate + 5 mL purified isopropyl ether (Caution! Isopropyl ether readily forms explosive peroxides!), shake at 100 strokes/min for 15 min, centrifuge at 2500 rpm for 10 min. Remove a 4.5 mL aliquot of the organic layer and add it to 300 μ L 10 mM HCl, shake at 150 strokes/min for 15 min, centrifuge at 2500 rpm for 10 min, discard the organic phase, vortex the aqueous layer under vacuum for 30 min (to remove traces of isopropyl ether), inject a 50 μ L aliquot of the aqueous layer. Urine. 100 μ L Urine + 1 mL 750 ng/mL IS in water + 200 μ L saturated sodium borate + 5 mL purified isopropyl ether, shake at 100 strokes/min for 15 min, centrifuge at 2500 rpm for 10 min. Remove a 4.5 mL aliquot of the organic layer and add it to 300 μ L 10 mM HCl, shake at 150 strokes/min for 15 min, centrifuge at 2500 rpm for 10 min, discard the organic phase, vortex the aqueous layer under vacuum for 30 min (to remove traces of isopropyl ether), inject a 50 μ L aliquot of the aqueous layer. (Purify isopropyl ether each day by passing it through a column of neutral alumina (Brockman Activity 1, Fisher).)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Supelcosil LC-8DB

Mobile phase: MeCN:100 mM pH 4.4 ammonium phosphate 25.5:74.5 (plasma) or 20:80 (urine)

Flow rate: 1

Injection volume: 50

Detector: UV 229

CHROMATOGRAM

Retention time: 10.1 (plasma), 12.2 (urine)

Internal standard: 1-[2-(dimethylamino)-1-(2-chlorophenyl)ethyl]cyclohexanol(Wy-45,818) (14.8 (plasma), 18.1 (urine))

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; dog; human; mouse; plasma; pharmacokinetics

REFERENCE

Hicks, D.R.; Wolaniuk, D.; Russell, A.; Cavanaugh, N.; Kraml, M. A high-performance liquid chromatographic method for the simultaneous determination of venlafaxine and *O*-desmethylvenlafaxine in biological fluids. *Ther.Drug Monit.*, **1994**, *16*, 100–107

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 1 mL pH 4.6 sodium acetate buffer + 200 μ L Glusulase, heat at 37° overnight, add MeCN, filter. Evaporate to dryness, reconstitute with 100 μ M (NH₄)₂PO₄, inject an aliquot.

HPLC VARIABLES

Guard column: 30 \times 4.6 10 μ m Spheri-10

Column: 250 \times 4.6 5 μ m LC-18-DB (Supelco)

Mobile phase: Gradient. MeCN:100 mM (NH₄)₂PO₄ from 10:90 to 40:60 over 25 min.

Flow rate: 1

Detector: UV 229

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

mouse; human

REFERENCE

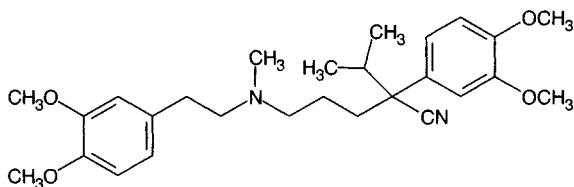
Howell, S.R.; Husbands, G.E.M.; Scatina, J.A.; Sisenwine, S.F. Metabolic disposition of ¹⁴C-venlafaxine in mouse, rat, dog, rhesus monkey and man. *Xenobiotica*, **1993**, *23*, 349–359

Verapamil

Molecular formula: C₂₇H₃₈N₂O₄

Molecular weight: 454.6

CAS Registry No.: 52-53-9 (verapamil),
152-11-4 (verapamil hydrochloride)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 250 μ L water + 5 mL MTBE, shake for 10 min, centrifuge at 1800 g for 10 min. Remove a 4 mL aliquot of the organic layer and add it to 150 μ L 17 mM phosphoric acid, shake for 3 min, centrifuge at 2800 g for 10 min, inject a 50 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 \times 4 10 μ m Spherisorb ODS 2

Mobile phase: MeCN:buffer 60:40 (Buffer was 1.15 g/L (NH₄)₂PO₄ containing 0.6 mL/L triethylamine, adjusted to pH 3.7 with phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 238

CHROMATOGRAM

Retention time: 13.6

Internal standard: verapamil

OTHER SUBSTANCES

Extracted: diltiazem

KEY WORDS

plasma; verapamil is IS

REFERENCE

Coors, C.; Schulz, H.-G.; Stache, F. Development and validation of a bioanalytical method for the quantification of diltiazem and desacetyldiltiazem in plasma by capillary zone electrophoresis. *J.Chromatogr.A*, **1995**, 717, 235-243

SAMPLE

Matrix: blood

Sample preparation: Add 200 μ L whole blood to 400 μ L MeCN while mixing rapidly for 5 s, centrifuge at 1500 rpm for 2 min, mix 200 μ L supernatant with 700 μ L 20 mM tetramethylammonium perchlorate containing 0.1% trifluoroacetic acid, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 50 \times 4.6 Little Champ reverse phase (Regis)

Mobile phase: MeCN:MeOH:buffer 37:5:58 (Buffer was 20 mM tetramethylammonium perchlorate containing 0.1% trifluoroacetic acid.)

Flow rate: 1.4

Injection volume: 50

Detector: UV 228

CHROMATOGRAM

Limit of quantitation: 100 nM

KEY WORDS

rat; whole blood

REFERENCE

Hoffman, D.J.; Seifert, T.; Borre, A.; Nellans, H.N. Method to estimate the rate and extent of intestinal absorption in conscious rats using an absorption probe and portal blood sampling. *Pharm.Res.*, **1995**, *12*, 889–894

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L 2.5 μ g/mL (+)-glaucine + 100 μ L water + 200 μ L 2 M NaOH, mix, add 6 mL hexane: sec-butanol 998:2, vortex for 10 min, centrifuge at 1500 g for 10 min, freeze in dry ice/acetone. Remove the hexane layer, add 50 μ L acetic anhydride, mix, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μ L MeCN:10 mM pH 4.8 potassium phosphate buffer 10:90, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 3 Chiral-AGP (ASTEC, Whippany, NJ)

Column: 150 \times 4 Chiral-AGP (ASTEC, Whippany, NJ)

Mobile phase: MeCN:10 mM pH 6.65 potassium phosphate buffer

Flow rate: 0.9

Injection volume: 100

Detector: F ex 227 em 308

CHROMATOGRAM

Retention time: 27 (R), 35 (S)

Internal standard: (+)-glaucine (20)

Limit of quantitation: 3.2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, norverapamil

KEY WORDS

plasma; chiral

REFERENCE

Stagni, G.; Gillespie, W.R. Simultaneous analysis of verapamil and norverapamil enantiomers in human plasma by high-performance liquid chromatography. *J.Chromatogr.B*, **1995**, *667*, 349–354

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 μ m 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: 5.38
Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acenocoumarol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benazepril, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, caffeine, carbamazepine, carpipramine, carteolol, celiprolol, chlorambucil, chlormezanone, chlorophenacinone, chloroquine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenoprofen, fentiazac, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephenesin, mephentermine, mepivacaine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metoclopramide, metoprolol, mexiletine, mianserine, minoxidil, moclobemide, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, penbutolol, penfluridol, pentazocine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thioproperazine, thioridazine, tiapride, tiaprofenic acid, ticlopidine, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, tropatenine, viloxazine, vinblastine, vincristine, vandesine, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: bupivacaine, buprenorphine, buspirone, carbinoxamine, cetirizine, chlordiazepoxide, chlorpheniramine, clorazepate, fenfluramine, flecainide, loprazolam, metapramine, metipranolol, midazolam, moperone, naproxen, oxprenolol, phencyclidine, thiopental, tianeptine, triprolidine, warfarin

KEY WORDS

whole blood; plasma

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 + 20 μ L 10 μ g/mL IS in water + 500 μ L 1 M NaOH + 5 mL diethyl ether, shake horizontally for 20 min, centrifuge at 1000 g for 10 min.

Remove the organic layer and add it to 14 mL 50 mM sulfuric acid, shake for 20 min, centrifuge at 400 g, discard the ether layer, remove traces of ether from the aqueous layer at 20°, inject a 100 µL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.5 µm CGC SGX18 (Tessek)

Mobile phase: MeOH:water:concentrated sulfuric acid 97:3:0.005

Flow rate: 0.4

Injection volume: 100

CHROMATOGRAM

Internal standard: D-517-HCL

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: norverapamil

KEY WORDS

serum; pharmacokinetics

REFERENCE

Vlcek, J.; Macek, K.; Hulek, P.; Brátová, M.; Fendrich, Z. Pharmacokinetic parameters of verapamil and its active metabolite norverapamil in patients with hepatopathy. *Arzneimittelforschung*, **1995**, *45*, 146–149

SAMPLE

Matrix: blood

Sample preparation: 100 µL Plasma + 375 µL MeCN + 25 µL 1 mg/mL 5,6-benzoquinoline in mobile phase, vortex for 13 min, centrifuge at 5000 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.5 µm Ultracarb C20 ODS (Phenomenex)

Mobile phase: MeCN:0.07% orthophosphoric acid 33:67

Flow rate: 1

Injection volume: 5

Detector: F ex 280 em 320

CHROMATOGRAM

Retention time: 10

Internal standard: 5,6-benzoquinoline (13)

Limit of detection: 3 ng/mL

KEY WORDS

plasma; rabbit; pharmacokinetics

REFERENCE

Yazan, Y.; Bozan, B. Rapid analysis of verapamil in plasma by reversed phase HPLC. *Pharmazie*, **1995**, *50*, 117–119

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 µm CN endcapped SPE cartridge with 1 mL MeOH then 1 mL buffer. 1.5 mL Plasma + 30 µL 5 µg/mL gallopamil in water, mix, add 1 mL to the SPE cartridge, wash with 1 mL buffer, elute with 240 µL

MeOH:2-aminoheptane 99.8:0.2, elute with 410 μ L pH 3.0 acetate buffer (from mobile phase), mix eluates, inject a 250 μ L aliquot. (Buffer was 250 mL 100 mM KH_2PO_4 + 195.5 mL 100 mM NaOH, make up to 1 L, pH 7.4.)

HPLC VARIABLES

Guard column: 4 \times 4 LiChroCART 5 μ m LiChrospher 100 RP-18

Column: 250 \times 4 LiChroCART 4 μ m Superspher 100 RP-18

Mobile phase: MeCN:2-aminoheptane:buffer 30:0.5:70 (Buffer was 33 mL glacial acetic acid in water, pH adjusted to 3.0 with 0.01 M NaOH.)

Column temperature: 35

Flow rate: 1.1

Injection volume: 250

Detector: F ex 275 em 310

CHROMATOGRAM

Retention time: 11

Internal standard: gallopamil (13)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: norverapamil

KEY WORDS

plasma; SPE; rugged

REFERENCE

Hubert, P.; Crommen, J. HPLC determination of verapamil and norverapamil in plasma using automated solid phase extraction for sample preparation and fluorometric detection. *J.Liq.Chromatogr.*, 1994, 17, 2147-2170

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 the SPE cartridge, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μ L 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 μ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)

Column: 150 \times 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: 4.8

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, 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, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: imipramine, maprotiline, nortriptyline

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: 1 mL Plasma + 40 μ L 20 μ g/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 μ L 5 mM sulfuric acid, vortex for 30 s, shake for 10 min, centrifuge at 700 g for 5 min, inject a 40 μ L aliquot of the aqueous layer.

HPLC VARIABLES

Column: 300 \times 3.9 10 μ m μ Bondapak 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: 15.5

Internal standard: deacetyldiltiazem (7)

Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Simultaneous: celiprolol, diltiazem, propranolol

Noninterfering: atenolol, aspirin, caffeine, ibuprofen, lidocaine, metoprolol, nifedipine

Interfering: desipramine

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: 1 mL Plasma + 1 mL 0.9% NaCl + 120 μ L 2 M NaOH + 5 mL n-hexane, vortex for 30 s, mix for 10 min, centrifuge at 2500 g for 20 min, repeat extraction with 4 mL n-hexane. Combine the organic phases and evaporate them to dryness, rinse walls with 1 mL n-hexane, evaporate to dryness, dissolve residue in 50 μ L isopropanol, inject a 25 μ L aliquot.

HPLC VARIABLES

Guard column: 50 \times 4.6 Chiralpak AD (Baker)

Column: 250 \times 4.6 Chiralpak AD (Baker)

Mobile phase: n-Hexane:isopropanol 90:10 with 0.1% diethylamine

Flow rate: 1

Injection volume: 25

Detector: F ex 223 no emission filter

CHROMATOGRAM

Retention time: 10 (S(-)), 12 (R(+))

Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: norverapamil

Also analyzed: gallopamil

KEY WORDS

plasma; chiral

REFERENCE

Fieger, H.; Blaschke, G. Direct determination of the enantiomeric ratio of verapamil, its major metabolite norverapamil, and gallopamil in plasma by chiral high-performance liquid chromatography. *J.Chromatogr.*, **1992**, *575*, 255–260

SAMPLE

Matrix: blood

Sample preparation: 1 mL plasma + 50 μ L 400 ng/mL (+)-glaucine + 100 μ L 2 M NaOH + 1 mL pH 7.0 sodium phosphate buffer (ionic strength 1) + 6 mL heptane, vortex for 1 min, centrifuge at 1500 g for 10 min, freeze in a dry ice bath. Decant heptane layer and evaporate it to dryness in a vacuum centrifuge at 60°, reconstitute the residue in 250 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Guard column: 50 \times 4.5 μ m LiChrocart DIOL (column temp ambient)

Column: 250 \times 4.6 10 μ m Chiralpak AD (column temp 30°)

Mobile phase: Hexane:isopropanol:ethanol 85:7.5:7.5 containing 1% triethylamine

Column temperature: 30

Flow rate: 1

Injection volume: 100

Detector: F ex 272 em 317

CHROMATOGRAM

Retention time: 7 (S), 8 (R)

Internal standard: (+)-glaucine (9.5)

Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, norverapamil

KEY WORDS

plasma; chiral

REFERENCE

Shibukawa, A.; Wainer, I.W. Simultaneous direct determination of the enantiomers of verapamil and norverapamil in plasma using a derivatized amylose high-performance liquid chromatographic chiral stationary phase. *J.Chromatogr.*, **1992**, *574*, 85–92

SAMPLE

Matrix: blood

Sample preparation: Add n-propyl p-aminobenzoate to plasma, deproteinize 1 mL plasma with 1 mL MeCN, inject an aliquot onto column A with mobile phase A, monitor effluent from column A with detector A, allow eluate containing verapamil to fill a 2 mL sample loop for 2 min, elute contents of sample loop onto column B with mobile phase B and at the same time allow pure mobile phase B to flow onto column B (ratio of column loop flow: pure mobile phase B flow 1:9), elute contents of column B onto column C with mobile phase C, monitor effluent from column C with detector B.

HPLC VARIABLES

Column: A 150 × 4.6 Inertsil ODS-2; B 10 × 4 Ultron ES-OVMG; C 150 × 4.6 Ultron ES-OVM

Mobile phase: A MeCN:water 30:70 containing 5 mM sodium 1-pentanesulfonate, adjust pH to 3.0 with phosphoric acid; B 5 mM pH 7.5 phosphate buffer; C THF:EtOH:water 1:8:91 containing 20 mM KH_2PO_4

Flow rate: A 1; B 4; C 1

Detector: UV 230 (A); UV 230 (B)

CHROMATOGRAM

Retention time: 14 (from column A), 23.5 (l enantiomer from column C), 25 (d enantiomer from column C)

Internal standard: n-propyl p-aminobenzoate (17, from column A)

Limit of detection: 1 ng/mL

KEY WORDS

plasma; column-switching; chiral

REFERENCE

Oda, Y.; Asakawa, N.; Kajima, T.; Yoshida, Y.; Sato, T. On-line determination and resolution of verapamil enantiomers by high-performance liquid chromatography with column switching. *J.Chromatogr.*, **1991**, *541*, 411–418

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 250 μL MeCN + 20 mg MgSO_4 + 10 mg ZnSO_4 , vortex for 3 min, centrifuge at 2000 g for 6 min. Remove the supernatant, filter (0.45 μm), inject a 100 μL aliquot. (Tubes were cleaned by sonication with acetone for 30 min, sonication twice with MeCN for 15 min, and drying at 100°. Stir 100 g ZnSO_4 crystals with 200 mL MeCN for 30 min, decant MeCN, repeat twice with fresh MeCN, dry crystals in a hood at room temperature.)

HPLC VARIABLES**Column:** 80 × 2.5 μm Spherisorb C8**Mobile phase:** MeCN:10 mM KH₂PO₄ 60:40, pH adjusted to 7.1 with 100 mM NaOH**Flow rate:** 0.8**Injection volume:** 100**Detector:** UV 220

CHROMATOGRAM**Retention time:** 8**Limit of quantitation:** 30 ng/mL

KEY WORDSplasma

REFERENCERustum, A.M. Measurement of verapamil in human plasma by reversed-phase high-performance liquid chromatography using a short octyl column. *J.Chromatogr.*, **1990**, *528*, 480–486

SAMPLE**Matrix:** blood**Sample preparation:** 500 μL Serum + 250 μL diisopropyl ether:n-butyl alcohol 7:3 containing 400 ng/mL minaprine (Caution! Diisopropyl ether readily forms explosive peroxides!), centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES**Guard column:** 30 × 4.6 μm Brownlee cyano spheri-5**Column:** 250 × 4.6 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 235

CHROMATOGRAM**Retention time:** 6**Internal standard:** minaprine (5.5)**Limit of detection:** 20 ng/mL

OTHER SUBSTANCES**Also analyzed:** amiodarone, amitriptyline, clomipramine, desipramine, diltiazem, haloperidol, imipramine, nortriptyline, propafenone

KEY WORDSserum

REFERENCEMazzi, 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:** formulations**Sample preparation:** Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 μm Spherisorb Phenyl

Mobile phase: MeCN:water:100 mM tetrabutylammonium hydrogen sulfate:500 mM KH_2PO_4 15:50:25:10, pH 5.1
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: amrinone

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

SAMPLE

Matrix: formulations

Sample preparation: 1 mL Sample + 9 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb phenyl

Mobile phase: MeCN:500 mM KH_2PO_4 :100 mM tetrabutylammonium hydrogen sulfate: water 25:10:25:40 adjusted to pH 5.4 with 10 M NaOH

Flow rate: 2

Injection volume: 20

Detector: UV 268

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: milrinone

KEY WORDS

stability-indicating; 5% dextrose; injections

REFERENCE

Riley, C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection. *Am.J.Hosp.Pharm.*, **1988**, 45, 2079–2091

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 65:35

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 7.1

Limit of detection: 1 $\mu\text{g/mL}$

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 \times 4.6 Chirex 3022 (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 280

KEY WORDS

chiral; $\alpha = 1.11$

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 \times 4.6 5 μm Supelcosil LC-DP (A) or 250 \times 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: 13.28 (A), 6.96 (B)

OTHER SUBSTANCES

Also analyzed: metabolites, 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, fenopropfen, 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, methyl dopa, 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, sulfonpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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: Isolate verapamil using achiral preparative HPLC, freeze dry an appropriate aliquot of the mobile phase, inject an aliquot into this system.

HPLC VARIABLES

Column: 150 × 4.6 OVM ovomucoid protein (Mac-Mod Analytical)

Mobile phase: EtOH:buffer 10:90 (Buffer was 13 mM KH₂PO₄ adjusted to pH 7.0 with phosphoric acid.)

Flow rate: 1

Detector: F ex 203 em 270 (cut-off)

CHROMATOGRAM

Retention time: 12.6 (S), 16.2 (R)

OTHER SUBSTANCES

Also analyzed: metabolites

KEY WORDS

chiral

REFERENCE

Lankford, S.M.; Bai, S.A. Determination of the stereochemical composition of the major metabolites of verapamil in dog urine with enantioselective liquid chromatographic techniques. *J.Chromatogr.B*, **1995**, *663*, 91–101

SAMPLE**Matrix:** solutions**Sample preparation:** Inject a 50-200 μ L aliquot of a solution in pH 7.4 Tyrode's buffer.

HPLC VARIABLES**Column:** 150 \times 3.9 4 μ m 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:** chlorpromazine, propantheline

KEY WORDSbuffer

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

HPLC VARIABLES**Column:** 150 \times 3.9 Nova-Pak C18**Mobile phase:** MeCN:buffer 35:65 containing 0.1% triethylamine (Buffer was 40 mM pH 4.0 phosphate buffer.)**Detector:** UV 230

REFERENCE

Surakitbanharn, Y.; McCandless, R.; Krzyzaniak, J.F.; Dannenfelser, R.-M.; Yalkowsky, S.H. Self-association of dexverapamil in aqueous solution. *J.Pharm.Sci.*, **1995**, *84*, 720-723

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Guard column:** 30 \times 3.2 7 μ m SI 100 ODS (not commercially available)**Column:** 150 \times 3.2 7 μ m 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; UV 274

CHROMATOGRAM**Retention time:** 4.1**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, 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 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, bromazepam, 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, dapson, 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, fenpropfen, 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, isocarboxystyryl, 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, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phency-

clidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phen-suximide, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, 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, tripeleppamine, triprolidine, tropacocaine, tyramine, 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 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: 10.29

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, diltiazem, metoprolol, nifedipine, prazosin, propranolol

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: 250 × 4.6 cellulose tris(3,5-difluorophenylcarbamate)

Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1

Flow rate: 0.5

Detector: UV

CHROMATOGRAM

Retention time: 56 (+), 66 (-)

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

Sample preparation: Prepare a 10 $\mu\text{g}/\text{mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 \times 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, bamethane, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, 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, dipyrizidamole, 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, hyoscyne, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, mep-tazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, 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, phen-butrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phen-tolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pin-dolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pir-tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolin-tane, 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, thiorida-zine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycy-promine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimetho-

benzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, 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: urine

Sample preparation: Adjust pH of 2 mL urine to 11 with 5 M NaOH, extract twice with 10 mL pentane:dichloromethane 70:30. Combine the organic layers and evaporate to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L MeOH, inject a 100 μ L aliquot. (Deconjugate 2 mL urine with 500 μ L 100 mM pH 4.5 sodium acetate buffer and 100 μ L Glusulase (90000 U/mL β -glucuronidase and 1000 U/mL sulfatase, Du Pont), heat at 37° for 18 h, proceed as before.)

HPLC VARIABLES

Column: 250 \times 10 10 μ m C18 (Alltech)

Mobile phase: Gradient. A was MeCN. B was 50 mM ammonium acetate adjusted to pH 4.5 with acetic acid. A:B 0:100 to 70:30 over 70 min.

Flow rate: 3

Injection volume: 100

Detector: F ex 280 em 340 (cut-off)

CHROMATOGRAM

Retention time: 66

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

dog; preparative

REFERENCE

Lankford, S.M.; Bai, S.A. Determination of the stereochemical composition of the major metabolites of verapamil in dog urine with enantioselective liquid chromatographic techniques. *J.Chromatogr.B*, **1995**, 663, 91–101

ANNOTATED BIBLIOGRAPHY

Romanová, D.; Brandsteterová, E.; Králiková, D.; Bozeková, L.; Kriska, M. Determination of verapamil and its metabolites in plasma using HPLC. *Pharmazie*, **1994**, 49, 779–780 [plasma; serum; SPE; LOQ 50 ng/mL; fluorescence detection]

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Köppel, C.; Wagemann, A. Plasma level monitoring of D, L-verapamil and three of its metabolites by reversed-phase high-performance liquid chromatography. *J.Chromatogr.*, **1991**, 570, 229–234 [LOD 5 ng/mL; fluorescence detection]

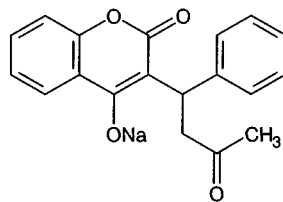
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Warfarin

Molecular formula: C₁₉H₁₆O₄

Molecular weight: 308.3

CAS Registry No.: 81-81-2 (warfarin), 129-06-6 (warfarin sodium),
2610-86-8 (warfarin potassium)



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + p-chlorowarfarin + 400 μ L 1 M sulfuric acid + 12 mL ethyl acetate, shake for 30 min, centrifuge at 1500 rpm for 10 min. Remove 10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μ L MeCN:water 57:43, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax C-8

Mobile phase: MeCN:water:glacial acetic acid 57:52.6:0.4

Flow rate: 1

Detector: F ex 310 em 370 following post-column reaction. The column effluent mixed with 12% triethanolamine (for fluorescence enhancement) pumped at 0.3 mL/min and the mixture flowed through a 2 mL reaction coil to the detector.

CHROMATOGRAM

Retention time: 9

Internal standard: p-chlorowarfarin (12)

Limit of quantitation: 6.3 ng/mL

KEY WORDS

plasma; post-column reaction; pharmacokinetics

REFERENCE

King, S.-Y.P.; Joslin, M.A.; Raudibaugh, K.; Pieniaszek, H.J., Jr.; Benedek, I.H. Dose-dependent pharmacokinetics of warfarin in healthy volunteers. *Pharm.Res.*, **1995**, *12*, 1874-1877

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 μ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μ L 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 \times 3.9 4 μ m NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ 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: 5.18

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acepromazine, aceprometazine, acetaminophen, aconitine, ajmaline, albuterol, alimemazine, alminoprofen, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amodiaquine, amoxapine, aspirin, astemizole, atenolol, benperidol, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, caffeine, carbamazepine, caripramine, carteolol, celiprolol, cetirizine, chlorambucil, chlormezanone, chlorphenacinone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, cicletanine, clemastine, clobazam, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, disopyramide, doxepine, doxepin, doxylamine, droperidol, ephedrine, estazolam, etodolac, fenoprofen, fentiazac, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrodine, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, ketoprofen, labetalol, levomepromazine, lidocaine, lidoflazine, lisinopril, loperamide, loprazolam, loratadine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mefenidramine, mefloquine, melphalan, meperidine, mephesisin, mephentermine, mepivacaine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metoclopramide, metoprolol, mianserine, minoxidil, moclobemide, moperone, morphine, nadolol, nalbuphine, naltrexone, naloxone, naltrexone, nialamide, nifedipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nomifensine, nortriptyline, omeprazole, opipramol, oxazepam, penbutolol, penfluridol, pentazocine, phenobarbital, phenol, phenylbutazone, pimozone, pindolol, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, secobarbital, sotalol, strychnine, sulfapyrazole, sulindac, sulpride, sultopride, suriclone, temazepam, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thioproperazine, thioridazine, tiapride, tiaprofenic acid, timolol, tiocloamarol, tofisopam, tolbutamide, toloxatone, triazolam, trifluoperazine, trifluoperidol, trimipramine, tropatenine, viloxazine, vinblastine, yohimbine, zolpidem, zopiclone, zorubicine

Interfering: acenocoumarol, benazepril, bupivacaine, buprenorphine, buspirone, carbinoxamine, chlordiazepoxide, clorazepate, dipyridamole, fenfluramine, flecainide, metapramine, metipranolol, mexiletine, midazolam, naproxen, oxprenolol, phencyclidine, pipamperone, pyrimethamine, thiopental, tianeptine, ticlopidine, trazodone, triprolidine, verapamil, vincristine, vindesine

KEY WORDS

whole blood; plasma

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 + 100 μ L 1 M HCl + 5 mL dichloromethane, stir for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, add 100 μ L 1 M (-)-1-menthylchloroformate in dichloromethane, add 20 μ L triethylamine, heat at 30° for 20 min, centrifuge, wash the supernatant with 3 mL 1 M HCl, evaporate to dryness, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Nucleosil silica

Mobile phase: Heptane:ethyl acetate 93:7 (water concentration 51 ppm)

Flow rate: 1

Injection volume: 50

Detector: UV 310

CHROMATOGRAM

Retention time: 10 (S), 11.5 (R)

KEY WORDS

derivatization; plasma; chiral; normal phase

REFERENCE

Aycard, M.; Letellier, S.; Maupas, B.; Guyon, F. Determination of (R) and (S) warfarin in plasma by high performance liquid chromatography using precolumn derivatization. *J.Liq.Chromatogr.*, **1992**, *15*, 2175-2182

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L 25 μ g/mL IS + 100 μ L 1 M HCl + 5 mL dichloromethane, stir 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 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Spherisorb ODS 2

Mobile phase: MeCN:MeOH:50 mM pH 4 acetate buffer 11:44:45

Flow rate: 1.5

Injection volume: 50

Detector: UV 310

CHROMATOGRAM

Internal standard: 8-chlorowarfarin

Limit of detection: 15 ng/mL

KEY WORDS

plasma

REFERENCE

Aycard, M.; Letellier, S.; Maupas, B.; Guyon, F. Determination of (R) and (S) warfarin in plasma by high performance liquid chromatography using precolumn derivatization. *J.Liq.Chromatogr.*, **1992**, *15*, 2175-2182

SAMPLE

Matrix: blood

Sample preparation: Prepare a chromatographic column of 250 mg silica gel H in a Pasteur pipette, wash with three 1 mL portions of diethyl ether and three 1 mL portions of hexane:ether 4:1. Dry 40-500 μ L 1 μ g/mL (+)-p-chlorowarfarin in MeOH in a tube under a stream of nitrogen, add 1 mL citrated plasma, add 100 μ L 1 M HCl, add 5 mL diethyl ether, vortex for 30 s, centrifuge at 1000 g for 5 min. Remove the ether layer and evaporate it at 40° under a stream of nitrogen, reconstitute in 1 mL hexane:ether 4:1, add to chromatographic column, wash with two 1 mL portions of hexane:ether 4:1, elute with 1 mL ether. Discard first 200 μ L eluate, evaporate remainder at 40°, add 15 μ L 50 mg/mL HCA in MeCN:water 25:1, add 10 μ L 100 mg/mL N, N'-dicyclohexylcarbodiimide in MeCN:water 25:1, let stand for 10 min, inject a 20 μ L aliquot within 4 h. (HCA was (-)-(1S,2R,4R)-endo-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2-carboxylic acid. Diethyl ether was freshly distilled before use.)

HPLC VARIABLES

Guard column: 15 × 4 10 μm LiChrosorb C18

Column: 250 × 4 10 μm LiChrosorb C18

Mobile phase: MeCN:water 80:20

Flow rate: 1.6

Injection volume: 20

Detector: F ex 313 em 370 (cut-off filter) following post-column reaction. The column effluent mixed with 200 mM NaOH pumped at 0.5 mL/min and the mixture flowed through a 1 m reaction coil to the detector.

CHROMATOGRAM

Retention time: 6 (S), 7 (R)

Internal standard: (+)-p-chlorowarfarin (9)

Limit of detection: 5 ng/mL

KEY WORDS

plasma; SPE; derivatization; chiral; post-column reaction

REFERENCE

Carter, S.R.; Duke, C.C.; Cutler, D.J.; Holder, G.M. Sensitive stereospecific assay of warfarin in plasma: reversed-phase high-performance liquid chromatographic separation using diastereoisomeric esters of (-)-(1S,2R,4R)-endo-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2-carboxylic acid. *J.Chromatogr.*, **1992**, *574*, 77-83

SAMPLE

Matrix: blood

Sample preparation: Acidify 1 mL plasma to pH <2 with 100 μL 2 M HCl, extract with 5 mL diethyl ether:hexane 50:50. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 20 μL 100 mg/mL carbobenzyloxy-L-proline in MeCN, 20 μL 1 mg/mL imidazole in MeCN, and 20 μL 100 mg/mL dicyclohexylcarbodiimide in MeCN, vortex for 10 s, let stand for 5-16 h, make up to 100 μL with MeCN, inject a 20 μL aliquot (J.Pharm.Sci. 1983, 72, 921; J.Clin.Pharmacol. 1995, 35, 1008).

HPLC VARIABLES

Column: 100 × 4.6 3 μm Microspher C18 (Chrompack)

Mobile phase: MeCN:isopropanol:pH 6.6 phosphate buffer (I=0.017) 36:12:52

Flow rate: 1.5

Injection volume: 20

Detector: UV 313

CHROMATOGRAM

Retention time: 14 (R), 17 (S)

Internal standard: p-chlorowarfarin (26)

Limit of detection: 60 ng/mL

KEY WORDS

plasma; chiral; derivatization; pharmacokinetics

REFERENCE

Sutfin, T.; Balmer, K.; Boström, H.; Eriksson, S.; Höglund, P.; Paulsen, O. Stereoselective interaction of omeprazole with warfarin in healthy men. *Ther.Drug Monit.*, **1989**, *11*, 176-184

SAMPLE

Matrix: blood

Sample preparation: Centrifuge serum at 15° at 203000 g for 20 h, remove 1 mL of the supernatant and add it to 10 μL 1.5 μg/mL naproxen in 100 mM NaOH, add 250 μL 4

M HCl, add 5 mL distilled diethyl ether, rotate at 30 rpm for 15 min, centrifuge at 700 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 μ L mobile phase, inject the whole amount.

HPLC VARIABLES

Column: μ Bondapak C18 Radial Pak

Mobile phase: MeCN:100 mM (NH₄)H₂PO₄ 63:37

Flow rate: 1.5

Injection volume: 100

Detector: F ex 320 em 390 following post-column reaction. The column effluent mixed with 12% triethanolamine (for fluorescence enhancement) pumped at 0.5 mL/min and the mixture flowed through a 1 m reaction coil to the detector.

CHROMATOGRAM

Internal standard: naproxen

Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES

Simultaneous: salicylic acid

Noninterfering: acetaminophen, carbamazepine, furosemide, hydrochlorothiazide, phenobarbital, phenytoin, spironolactone

KEY WORDS

serum; post-column reaction; ultracentrifugate

REFERENCE

Steyn, J.M.; van der Merwe, H.M.; de Kock, M.J. Reversed-phase high-performance liquid chromatographic method for the determination of warfarin from biological fluids in the low nanogram range. *J. Chromatogr.*, **1986**, *378*, 254–260

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma + 100 μ L 8.46 μ g/mL IS in water + 1 mL 100 mM potassium carbonate + 4 mL ether, shake for 3 min, centrifuge at 3000 rpm for 5 min, discard the organic layer. Acidify the aqueous layer with 1.5 mL 1 M HCl, add 6 mL ether, shake for 3 min, centrifuge at 3000 rpm for 3 min, freeze by immersion in liquid nitrogen for 40–60 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 10 μ L 100 mg/mL carbobenzyloxy-L-proline in MeCN, 10 μ L 1 mg/mL imidazole in MeCN, and 10 μ L 100 mg/mL dicyclohexylcarbodiimide in MeCN, vortex for 10 s, let stand for 2 h, centrifuge at 3000 rpm for 5 min, inject a 3–10 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 5 μ m Spherisorb Si

Mobile phase: Hexane:ethyl acetate:MeOH:acetic acid 74.75:25:0.25:0.4

Flow rate: 1

Injection volume: 3–10

Detector: UV 313

CHROMATOGRAM

Retention time: 14 (SS), 17 (RS)

Internal standard: 4'-fluorowarfarin (15, 19 (enantiomers))

Limit of detection: 160 ng (S); 96 ng (R)

KEY WORDS

plasma; chiral; derivatization; pharmacokinetics; normal phase

REFERENCE

Banfield, C.; Rowland, M. Stereospecific high-performance liquid chromatographic analysis of warfarin in plasma. *J.Pharm.Sci.*, **1983**, *72*, 921-924

SAMPLE

Matrix: cell suspensions

Sample preparation: Filter microbial cell suspensions. 4 mL Filtrate + 200 μ L water + 100 μ L 4 μ g/mL 4'-hydroxywarfarin alcohols (potassium salt) in water + 4 mL cyclohexane:dichloromethane 90:10, extract twice, discard organic layers. Extract aqueous layer with 5 mL ethyl acetate. Extract the organic phase with 2 mL 100 mM KOH. Acidify the aqueous phase with 1 mL 5 M HCl, extract with 5 mL diethyl ether. Evaporate the ether layer to dryness under a stream of nitrogen, reconstitute the residue in 250 μ L mobile phase, inject a 50-200 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.5 5 μ m Beckman ODS C18

Mobile phase: MeOH:THF:1 M aqueous tetrabutylammonium phosphate:5 mM ammonium phosphate adjusted to pH 7.5 with ammonium hydroxide 30:7:1:62

Flow rate: 1

Injection volume: 50-200

Detector: F ex 290 emission filter No. 389

CHROMATOGRAM

Retention time: 28

Internal standard: 4'-hydroxywarfarin alcohols (4, 6)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Wong, Y.W.; Davis, P.J. Analysis of warfarin and its metabolites by reversed-phase ion-pair liquid chromatography with fluorescence detection. *J.Chromatogr.*, **1989**, *469*, 281-291

SAMPLE

Matrix: formulations

Sample preparation: Dilute with saline, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 8 Novapak C18 Radial-Pak

Mobile phase: MeCN:1.5% acetic acid 31:69 adjusted to pH 4.2 with 1 M NaOH

Flow rate: 4.2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 14

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: formulations

Sample preparation: Powder tablets, dissolve in mobile phase, sonicate, dilute with mobile phase to give a 200 µg/mL solution, 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: dicumarol, 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

SAMPLE

Matrix: microsomal incubations

Sample preparation: 500 µL Microsomal incubation + 10 µL 70% perchloric acid + 10 ng 7-ethoxycoumarin, centrifuge at 4° at 3000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 11 × 4 10 µm Nucleosil C8

Column: 125 × 4 5 µm Nucleosil C18

Mobile phase: MeCN:0.5% phosphoric acid 36:62

Flow rate: 1.3

Injection volume: 100

Detector: UV 205

CHROMATOGRAM

Retention time: 11.7

Internal standard: 7-ethoxycoumarin (7)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver

REFERENCE

Lang, D.; Böcker, R. Highly sensitive and specific high-performance liquid chromatographic analysis of 7-hydroxywarfarin, a marker for human cytochrome P-4502C9 activity. *J.Chromatogr.B*, **1995**, *672*, 305–309

SAMPLE

Matrix: solutions

Sample preparation: Filter (0.45 µm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:10 mM pH 4.7 acetate buffer 50:50

Detector: UV 214

REFERENCE

Okimoto, K.; Rajewski, R.A.; Uekama, K.; Jona, J.A.; Stella, V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins. *Pharm.Res.*, **1996**, *13*, 256–264

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3022 (Phenomenex)

Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 88:10:2 (EtOH/trifluoroacetic acid was premixed 20:1.)

Flow rate: 0.7-1

Injection volume: 20

Detector: UV 282

KEY WORDS

chiral; $\alpha = 1.03$

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: 8.01 (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, 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, fenpropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvox-

amine, 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, methyl dopa, 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, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, xylometazoline, yohimbine, zopiclone

KEY WORDS

some 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: 100 × 4.5 μm Chiral-AGP (ChromTech)

Mobile phase: Isopropanol:20 mM pH 6.0 potassium phosphate buffer 15:85

Flow rate: 1

Detector: UV 205

KEY WORDS

chiral

REFERENCE

Lang, D.; Böcker, R. Highly sensitive and specific high-performance liquid chromatographic analysis of 7-hydroxywarfarin, a marker for human cytochrome P-4502C9 activity. *J.Chromatogr.B*, **1995**, 672, 305–309

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot. (Buffer was 100 mM pH 5.3 sodium acetate.)

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 bind-

ing 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 4.6 KH₂PO₄ 10:90

Flow rate: 0.8

Injection volume: 20

Detector: UV

CHROMATOGRAM

Retention time: k' 4.67

KEY WORDS

chiral; $\alpha = 1.39$

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica

Mobile phase: Heptane:isopropanol 90:10

Flow rate: 1

Injection volume: 1000

Detector: UV 254

CHROMATOGRAM

Retention time: k' 4.85

KEY WORDS

chiral; $\alpha = 1.81$

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: 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, bromazepam, 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, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, 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, mephentoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, nicotinic acid, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phen-suximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrillamine, 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, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, 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 MeOH, inject a 1-2 μ L aliquot.

HPLC VARIABLES

Guard column: 10 × 3 5 μm Chiral-AGP (ChromTech)

Column: 100 × 4 5 μm Chiral-AGP (ChromTech)

Mobile phase: Gradient. A was 10 mM pH 7.0 phosphate buffer containing 1 mM N, N-dimethyloctylamine. B was 1 mM N, N-dimethyloctylamine in isopropanol. A:B from 100:0 to 80:20 over 10 min, stay at 80:20 for 15 min, equilibrate at 100:0 for 10 min.

Flow rate: 0.9

Injection volume: 1-2

Detector: F ex 292 em 380

CHROMATOGRAM

Retention time: 9.4 (S), 10.4 (R)

OTHER SUBSTANCES

Simultaneous: acenocoumarol, phenprocoumon, warfarin alcohol

KEY WORDS

chiral

REFERENCE

de Vries, J.X.; Schmitz-Kummer, E. Direct column liquid chromatographic enantiomer separation of the coumarin anticoagulants phenprocoumon, warfarin, acenocoumarol and metabolites on an α1-acid glycoprotein chiral stationary phase. *J.Chromatogr.*, **1993**, *644*, 315–320

SAMPLE

Matrix: solutions

Sample preparation: Condition a 3 mL 200 mg Bond-Elut C18 SPE cartridge with 5 mL MeCN then 5 mL water (pH adjusted to 4.3 with acetic acid), do not allow to dry. Acidify water to pH 4.3 with acetic acid, run 1 L through the SPE cartridge with a vacuum, wash with 20 mL MeCN:water adjusted to pH 4.3 with acetic acid 20:80, elute with 1 mL MeCN:40 mM pH 7.4 phosphate buffer 1:1, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: MeOH:water:glacial acetic acid 62:38:1

Flow rate: 1

Injection volume: 30

Detector: UV 282; UV 306

CHROMATOGRAM

Retention time: 5

Limit of detection: 0.02 ppb

KEY WORDS

SPE; water

REFERENCE

Dalbacke, J.; Dahlquist, I.; Persson, C. Determination of warfarin in drinking water by high-performance liquid chromatography after solid-phase extraction. *J.Chromatogr.*, **1990**, *507*, 381–387

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)

Mobile phase: Hexane:isopropanol:formic acid 80:20:1

Flow rate: 0.5

Detector: UV

CHROMATOGRAM

Retention time: 17 (-), 36 (+)

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

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Haginaka, J.; Kanasugi, N. Enantioselectivity of bovine serum albumin-bonded columns produced with isolated protein fragments. *J.Chromatogr.A*, **1995**, *694*, 71–80 [chiral; also benzoin, clorazepate, fenopropfen, flurbiprofen, ibuprofen, ketoprofen, lorazepam, lormetazepam, oxazepam, pranoprofen, temazepam]

Terfloth, G.J.; Pirkle, W.H.; Lynam, K.G.; Nicolas, E.C. Broadly applicable polysiloxane-based chiral stationary phase for high-performance liquid chromatography and supercritical fluid chromatography. *J.Chromatogr.A*, **1995**, *705*, 185–194 [SFC; HPLC; also carprofen, cicloprofen, etodolac, fenopropfen, flurbiprofen, ibuprofen, naproxen, piroprofen]

Cai, W.M.; Hatton, J.; Pettigrew, L.C.; Dempsey, R.J.; Chandler, M.H.H. A simplified high-performance liquid chromatographic method for direct determination of warfarin enantiomers and their protein binding in stroke patients. *Ther Drug Monit.*, **1994**, *16*, 509–512 [chiral; fluorescence detection; phenprocoumon (IS); LOD 8 ng/mL; post-column reaction detection; derivatization]

Ermer, J.C.; Hicks, D.R.; Wheeler, S.C.; Kraml, M.; Jusko, W.J. Concomitant etodolac affects neither the unbound clearance nor the pharmacologic effect of warfarin. *Clin.Pharmacol.Ther.*, **1994**, *55*, 305–316 [serum; nitrazepam (IS); SPE; LOD 15 ng/mL; pharmacokinetics]

Loun, B.; Hage, D.S. Chiral separation mechanisms in protein-based HPLC columns. 1. Thermodynamic studies of (R)- and (S)-warfarin binding to immobilized human serum albumin. *Anal.Chem.*, **1994**, *66*, 3814–3822 [column temp 37]

Wang, J.-P.; Unadkat, J.D.; McNamara, S.; O'Sullivan, T.A.; Smith, A.L.; Trager, W.F.; Ramsey, B. Disposition of drugs in cystic fibrosis VI. In vivo activity of cytochrome P450 isoforms involved in the metabolism of (R)-warfarin (including P450 3A4) is not enhanced in cystic fibrosis. *Clin.Pharmacol.Ther.*, **1994**, *55*, 528–534 [plasma; fluorowarfarin (IS); LOQ 50 ng/mL; pharmacokinetics]

Naidong, W.; Lee, J.W. Development and validation of a high-performance liquid chromatographic method for the quantitation of warfarin enantiomers in human plasma. *J.Pharm.Biomed.Anal.*, **1993**, *11*, 785–792

Shibukawa, A.; Nagao, M.; Kuroda, Y.; Nakagawa, T. Stereoselective determination of free warfarin concentration in protein binding equilibrium using direct sample injection and an on-line liquid chromatographic system. *Anal.Chem.*, **1990**, *62*, 712–716 [chiral; column-switching; column temp 37]

de Vries, J.X.; Volker, U. Separation of the enantiomers of phenprocoumon and warfarin by high-performance liquid chromatography using a chiral stationary phase. Determination of the enantiomeric ratio of phenprocoumon in human plasma and urine. *J.Chromatogr.*, **1989**, *493*, 149–156

Chan, K.; Woo, K.S. Determination of warfarin in human plasma by high performance liquid chromatography. *Methods Find.Exp.Clin.Pharmacol.*, **1988**, *10*, 699–703

Chu, Y.Q.; Wainer, I.W. The measurement of warfarin enantiomers in serum using coupled achiral/chiral, high-performance liquid chromatography (HPLC). *Pharm.Res.*, **1988**, *5*, 680–683

Wang, J.; Bonakdar, M. Sensitive measurements of warfarin by high-performance liquid chromatography with electrochemical detection. *J.Chromatogr.*, **1987**, *415*, 432–437

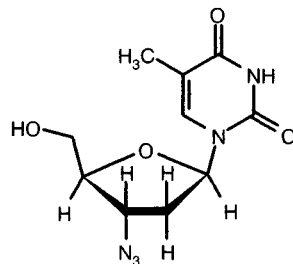
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- Banfield, C.; Rowland, M. Stereospecific fluorescence high-performance liquid chromatographic analysis of warfarin and its metabolites in plasma and urine. *J. Pharm. Sci.*, **1984**, *73*, 1392–1396
- Jeyaraj, G.L.; Porter, W.R. New method for the resolution of racemic warfarin and its analogues using low-pressure liquid chromatography. *J. Chromatogr.*, **1984**, *315*, 378–383
- Perez, R.L. Simultaneous determination of warfarin, sulphaquinoxaline and fenitrothion in wheat-based rodenticide baits by high pressure liquid chromatography. *J. Liq. Chromatogr.*, **1983**, *6*, 353–365
- Tasker, R.A.; Nakatsu, K. Rapid, reliable and sensitive assay for warfarin using normal-phase high-performance liquid chromatography. *J. Chromatogr.*, **1982**, *228*, 346–349
- Lee, S.H.; Field, L.R.; Howald, W.N.; Trager, W.F. High-performance liquid chromatographic separation and fluorescence detection of warfarin and its metabolites by postcolumn acid/base manipulation. *Anal. Chem.*, **1981**, *53*, 467–471
- Robinson, C.A.; Mungall, D.; Poon, M.C. Quantitation of plasma warfarin concentrations by high performance liquid chromatography. *Ther. Drug Monit.*, **1981**, *3*, 287–290
- Trujillo, W.A. Determination of warfarin and sulfaquinoxaline in rodenticide concentrates by HPLC. *J. Liq. Chromatogr.*, **1980**, *3*, 1219–1226

Zidovudine

Molecular formula: C₁₀H₁₃N₅O₄

Molecular weight: 267.2

CAS Registry No.: 30516-87-1



SAMPLE

Matrix: bile, blood, tissue

Sample preparation: Condition a Baker C18 SPE cartridge with three 1 mL portions of MeOH, three 1 mL portions of water, and three 1 mL portions of buffer. Add a 200 μ L aliquot of plasma, bile, or liver homogenate containing the IS to 200 μ L buffer and add this mixture to the SPE cartridge, wash with two 200 μ L portions of buffer, allow cartridge to dry, elute with two 100 μ L portions of MeOH, combine the eluates, add an equal volume of water, inject a 40 μ L aliquot. (Buffer was 200 mM pH 7.5 sodium phosphate containing 8 mM tetrabutylammonium sulfate.)

HPLC VARIABLES

Guard column: μ Bondapak C18 Guard-pak

Column: 150 \times 3.9 4 μ m Novapak C18

Mobile phase: MeCN:200 mM pH 7.5 sodium phosphate + 8 mM tetrabutylammonium sulfate 5:95 (Wash column overnight with MeOH.)

Flow rate: 1.5

Injection volume: 20-50

Detector: UV 254

CHROMATOGRAM

Retention time: 6.5

Internal standard: azidodddI

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: ddC, didanosine

KEY WORDS

plasma; liver; human; cat; rat; SPE

REFERENCE

Molema, G.; Jansen, R.W.; Visser, J.; Meijer, D.K.F. Simultaneous analysis of azidothymidine and its mono-, di- and triphosphate derivatives in biological fluids, tissue and cultured cells by a rapid high-performance liquid chromatographic method. *J.Chromatogr.*, **1992**, 579, 107-114

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 3 mL MeOH and 2 mL buffer, do not allow to go dry. 1 mL Plasma + 25 μ L 40 μ g/mL 7-ethyltheophylline in water, vortex, add to the SPE cartridge at not more than 0.5 mL/min, wash with 1 mL buffer, air dry for 3 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50 $^{\circ}$, reconstitute the residue in 100 μ L MeCN: 5% acetic acid 10:90, vortex vigorously, centrifuge at 10000 g for 5 min, inject a 20 μ L aliquot of the supernatant. (Prepare buffer by diluting 1.44 mL concentrated phosphoric acid to 1 L with water and adjusting the pH to 6.55 with concentrated ammonia.)

HPLC VARIABLES

Column: 75 \times 4.6 3 μ m Ultrasphere ODS

Mobile phase: Gradient. A was 1.44 mL concentrated phosphoric acid and 4 mL n-octylamine in 1 L water, pH adjusted to 6.55 with concentrated ammonia. B was MeCN. A:B from 95:5 to 70:30 over 7 min, to 20:80 over 1.5 min, return to initial conditions over 1 min, re-equilibrate for 2.5 min.

Flow rate: 1

Injection volume: 20

Detector: UV 266

CHROMATOGRAM

Retention time: 3.6

Internal standard: 7-ethyltheophylline (4.2)

Limit of detection: 7 ng/mL

Limit of quantitation: 22 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, caffeine

KEY WORDS

plasma; SPE

REFERENCE

Nadal, T.; Ortuño, J.; Pascual, J.A. Rapid and sensitive determination of zidovudine and zidovudine glucuronide in human plasma by ion-pair high-performance liquid chromatography. *J.Chromatogr.A*, **1996**, *721*, 127-137

SAMPLE

Matrix: blood

Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μ L serum while centrifuging at 17000 g for 1.5 h, inject a 50 μ L aliquot of the clear ultrafiltrate.

HPLC VARIABLES

Column: 150 \times 3.9 4 μ m 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: 22.4

OTHER SUBSTANCES

Extracted: didanosine, zalcitabine

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

Sample preparation: 100 μL Serum + 50 μL 5 $\mu\text{g}/\text{mL}$ IS + 50 μL 2 M perchloric acid, mix, centrifuge at 9000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:40 mM pH 7.0 sodium acetate 12:88

Flow rate: 2

Detector: UV 263

CHROMATOGRAM

Internal standard: 3'-azido-2',3'-dideoxy-5-ethyluridine (CS-85)

Limit of quantitation: 50 ng/mL

KEY WORDS

mouse; serum; pharmacokinetics

REFERENCE

Manouilov, K.K.; White, C.A.; Boudinot, F.D.; Fedorov, I.I.; Chu, C.K. Lymphatic distribution of 3'-azido-3'-deoxythymidine and 3'-azido-2',3'-dideoxyuridine in mice. *Drug Metab.Dispos.*, **1995**, 23, 655-658

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 400 μL ethyl acetate, 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 \times 4 5 μm Lichrospher 60 RP-select B

Column: 125 \times 4 5 μm Lichrospher 60 RP-select B

Mobile phase: MeOH:pH 7.0 phosphate buffer 20:80

Flow rate: 1

Injection volume: 10

Detector: UV 265

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: Heat blood at 56-58° for 30 min, add 500 μL serum to a lipophilic type W SPE cartridge (DuPont), wash with phosphate-buffered saline, elute with MeOH. Evaporate the eluate and reconstitute the residue with MeCN:water 15:85, inject an aliquot.

HPLC VARIABLES

Column: 5 μm Resolve C18 (Waters)

Mobile phase: MeCN:25 mM pH 2.20 phosphate buffer 15:85

Flow rate: 1

Detector: UV 266; UV 254

CHROMATOGRAM

Limit of detection: 40 nM

OTHER SUBSTANCES

Extracted: metabolites, glucuronylzidovudine

KEY WORDS

serum; pharmacokinetics; SPE

REFERENCE

Moore, K.H.P.; Raasch, R.H.; Brouwer, K.L.R.; Opheim, K.; Cheeseman, S.H.; Eyster, E.; Lemon, S.M.; van der Horst, C.M. Pharmacokinetics and bioavailability of zidovudine and its glucuronidated metabolite in patients with human immunodeficiency virus infection and hepatic disease (AIDS Clinical Trials Group Protocol 062). *Antimicrob. Agents Chemother.*, **1995**, 39, 2732–2737

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Serum + 10 μ L 500 ng/mL theophylline in water + 50 μ L isoamyl alcohol, vortex for 30 s, add 2 mL chloroform, vortex for 1 min, centrifuge at 1000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 5 μ m Novapak C18

Mobile phase: MeCN:0.2% acetic acid 7.5:92.5 (After 4 min increase flow rate to 1.8 mL/min over 1 min, maintain at 1.8 mL/min for 2 min.)

Flow rate: 0.8

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 6.0

Internal standard: theophylline (3.4)

Limit of detection: 20 ng/mL

KEY WORDS

rat; serum; pharmacokinetics

REFERENCE

Radwan, M.A. HPLC assay of theophylline and zidovudine in rat serum. *J. Liq. Chromatogr.*, **1995**, 18, 3301–3309

SAMPLE

Matrix: blood

Sample preparation: Plasma. 500 μ L Plasma + 500 μ L 35% perchloric acid, vortex for 1 min, centrifuge at 4000 g at 4° for 5 min. Remove a 200 μ L supernatant and add it to 800 μ L 250 ng/mL IS. Urine. Dilute urine 1:50 with water, add 200 μ L to 800 μ L 250 ng/mL IS, inject a 200 μ L aliquot. Purify on column A for 2 min then pass onto column B (?).

HPLC VARIABLES

Column: A 30 \times 3.9 40 μ m Perisorb RP8; B 20 \times 2 40 μ m Bondapak C18 endcapped + 300 \times 3 4 μ m Novapak RP18 endcapped

Mobile phase: MeOH:THF:25 mM pH 3.1 phosphate buffer 3.7:2.8:93.5

Flow rate: A 0.7; B 1

Injection volume: 200

Detector: UV 270

CHROMATOGRAM

Retention time: 16.8

Internal standard: 2-O-isopropylidene uridine (14)

Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Schrive, I.; Plasse, J.C. Quantification of zidovudine and one of its metabolites in plasma and urine by solid-phase extraction and high-performance liquid chromatography. *J.Chromatogr.B*, **1994**, 657, 233–237

SAMPLE

Matrix: blood

Sample preparation: 200 μ L Plasma + 50 μ L 20% w/v trichloroacetic acid, mix, heat at 60° for 1 h, centrifuge at 2500 g for 2 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.5 μ m reversed-phase C18 (FSA Laboratory Supplies)

Mobile phase: MeCN:20 mM pH 2.7 KH₂PO₄ 15:85

Flow rate: 0.4

Detector: UV 267

CHROMATOGRAM

Retention time: 16.8

Internal standard: β -hydroxyethyltheophylline (11)

Limit of quantitation: 430 nM

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: acetaminophen

Noninterfering: aspirin, fluconazole, ketoconazole, sulfamethoxazole, trimethoprim

KEY WORDS

plasma

REFERENCE

Kamali, F.; Rawlins, M.D. Simple and rapid assay for zidovudine and zidovudine glucuronide in plasma using high-performance liquid chromatography. *J.Chromatogr.*, **1990**, 530, 474–479

SAMPLE

Matrix: blood, cell suspensions, perfusate

Sample preparation: Centrifuge cellular suspensions at 17000 g for 5 min, inject a 25 μ L aliquot. Centrifuge perfusate at 17000 g for 5 min, inject a 50 μ L 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 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4.5 μ m Phenyl Hypersil NC-04

Mobile phase: MeOH:1.4 g/L sodium acetate 20:80, adjusted to pH 6.55

Flow rate: 1

Injection volume: 25-50

Detector: UV 267

CHROMATOGRAM

Retention time: 8

Limit of detection: 50 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, CSF

Sample preparation: Mix 100 μ L plasma or 50 μ L CSF with an equal volume of 5 μ g/mL 7- β -hydroxypropyltheophylline in MeCN, centrifuge at 14000 rpm, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 250 \times 4 LiChrospher RP-18e

Mobile phase: MeCN:water:acetic acid 15:84.9:0.1

Flow rate: 1

Injection volume: 20

Detector: UV 265

CHROMATOGRAM

Internal standard: 7- β -hydroxypropyltheophylline

KEY WORDS

rat; plasma; pharmacokinetics

REFERENCE

Seki, T.; Sato, N.; Hasegawa, T.; Kawaguchi, T.; Juni, K. Nasal absorption of zidovudine and its transport to cerebrospinal fluid in rats. *Biol.Pharm.Bull.*, **1994**, 17, 1135-1137

SAMPLE

Matrix: blood, milk, tissue

Sample preparation: Tissue. Homogenize 1 g tissue with 10 mL water. Add 2 mL MeOH to 1 mL tissue homogenate, mix, incubate at 4° for 1 h, centrifuge at 4°, put supernatant in a fresh tube, centrifuge, add p-nitrophenol to the supernatant, inject a 10-50 μ L aliquot. Serum. 1 mL Serum + 2 mL MeOH, mix, incubate at 4° for 1 h, centrifuge at 4°, add p-nitrophenol to the supernatant, inject a 10-50 μ L aliquot. Milk. 1 mL Milk + 4 mL MeOH, mix, incubate at 4° for 1 h, centrifuge at 4°, add p-nitrophenol to the supernatant, inject a 10-50 μ L aliquot.

HPLC VARIABLES

Guard column: 10 μ m C18 Guard-Pak

Column: 100 \times 5 5 μ m Radial-Pak NovA C18

Mobile phase: MeCN:100 mM ammonium acetate 6:94, adjusted to pH 4.5 with glacial acetic acid

Flow rate: 1

Injection volume: 10-50

Detector: UV 280

CHROMATOGRAM

Retention time: 20

Internal standard: p-nitrophenol (37)

Limit of detection: 150 ng/g

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; mouse; brain; embryonic tissue; spleen; liver

REFERENCE

Ruprecht, R.M.; Sharpe, A.H.; Jaenisch, R.; Trites, D. Analysis of 3'-azido-3'-deoxythymidine levels in tissues and milk by isocratic high-performance liquid chromatography. *J.Chromatogr.*, **1990**, 528, 371-383

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize (Polytron Model PT-1200C) 1 mL whole blood or whole rat brain with 1 mL water for 3-5 min, add 4 mL MeCN, vortex, add 1 mL concentrated saline, allow to settle at -5° for 1 h. Remove the organic layer, filter, dilute 1:4 with 50 mM ammonium acetate, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Adsorbosphere C18

Mobile phase: MeCN:50 mM ammonium acetate:water 20:55:25 adjusted to pH 5.5 with glacial acetic acid

Flow rate: 1

Detector: UV 266

CHROMATOGRAM

Retention time: 5.41

Limit of detection: 10-50 ng/mL

Limit of quantitation: 30-180 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; rat; brain; pharmacokinetics

REFERENCE

Brewster, M.E.; Andersom, W.R.; Helton, D.O.; Bodor, N.; Pop, E. Dose-dependent brain delivery of zidovudine through the use of a zidovudine chemical delivery system. *Pharm.Res.*, **1995**, 12, 796-798

SAMPLE

Matrix: cell media

Sample preparation: Inject a 150 µL aliquot of intracellular or extracellular media.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Partisil ODS3

Mobile phase: Gradient. MeCN:50 mM pH 6.5 phosphate buffer from 5:95 to 90:10 over 32 min

Flow rate: 1

Injection volume: 150

Detector: Radioactivity

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

liver; monkey; dog; rat; human

REFERENCE

Nicolas, F.; De Sousa, G.; Thomas, P.; Placidi, M.; Lorenzon, G.; Rahmani, R. Comparative metabolism of 3'-azido-3'-deoxythymidine in cultured hepatocytes from rats, dogs, monkeys, and humans. *Drug Metab. Dispos.*, **1995**, *23*, 308–313

SAMPLE

Matrix: cell suspensions

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

HPLC VARIABLES

Column: Partisil-10 SAX

Mobile phase: Gradient. 10 mM pH 3.6 ammonium phosphate:600 mM pH 3.8 ammonium phosphate 100:0 for 15 min then a convex gradient to 0:100 over 10 min then stay at 0:100 for 30 min.

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 6.5

OTHER SUBSTANCES

Extracted: metabolites

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: dialysate

Sample preparation: Vortex dialysate and IS, inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 200 \times 2.1 5 μ m Hypersil ODS

Mobile phase: MeCN:10 mM (NH₄)H₂PO₄ 15:85

Flow rate: 0.2

Injection volume: 5

Detector: UV 266

CHROMATOGRAM

Internal standard: β -hydroxypropyltheophylline

KEY WORDS

rabbit; pharmacokinetics

REFERENCE

Wang, Y.; Sawchuk, R.J. Zidovudine transport in the rabbit brain during intravenous and intracerebroventricular infusion. *J.Pharm.Sci.*, **1995**, *84*, 871–876

SAMPLE

Matrix: intestinal mucosal homogenate

Sample preparation: Homogenate mixture + 100 μ L 250 mM NaCN, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μ m) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 150 \times 3.9 Nova-Pak C18

Mobile phase: MeOH:100 mM potassium phosphate 25:75

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: solutions

Sample preparation: Inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 200 \times 4.6 5 μ m HP Hypersil ODS

Mobile phase: MeCN:20 mM pH 7.0 Na₂HPO₄ 20:80

Column temperature: 37

Flow rate: 1

Injection volume: 15

Detector: UV 265

CHROMATOGRAM

Retention time: 4.18

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

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1 mg/mL solution in water, inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 7 μ m Hypercarb (Shandon)

Mobile phase: THF:water:trifluoroacetic acid 30:69.5:0.5

Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: metabolites, zidovudine glucuronide

REFERENCE

Ayrton, J.; Evans, M.B.; Harris, A.J.; Plumb, R.S. Porous graphitic carbon shows promise for the rapid chromatographic analysis of polar drug metabolites. *J.Chromatogr.B*, **1995**, 667, 173–178

SAMPLE

Matrix: solutions
Sample preparation: Inject a 15 μ L aliquot.

HPLC VARIABLES

Column: 200 \times 4.6 5 μ m HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na₂HPO₄ 20:80
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM

Retention time: 4.18

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: tissue
Sample preparation: 50 mg Lymph nodes + 50 μ L 10 μ g/mL IS, homogenize with 6 volumes of pH 7.4 phosphate buffer, add 3 volumes of MeCN, mix, centrifuge at 9000 rpm for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 22°, reconstitute the residue in 150 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Hypersil ODS
Mobile phase: MeCN:buffer 10:90 (Buffer was 0.3% acetic acid adjusted to pH 4.0 with triethylamine.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 263

CHROMATOGRAM

Internal standard: 3'-azido-2',3'-dideoxy-5-ethyluridine (CS-85)
Limit of quantitation: 50 ng/g

KEY WORDS

mouse; lymph nodes; pharmacokinetics

REFERENCE

Manouilov, K.K.; White, C.A.; Boudinot, F.D.; Fedorov, I.I.; Chu, C.K. Lymphatic distribution of 3'-azido-3'-deoxythymidine and 3'-azido-2',3'-dideoxyuridine in mice. *Drug Metab.Dispos.*, **1995**, *23*, 655–658

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Almudaris, A.; Ashton, D.S.; Ray, A.; Valko, K. Trace analysis of impurities in 3'-azido-3'-deoxythymidine by reversed-phase high-performance liquid chromatography and thermospray mass spectrometry. *J.Chromatogr.A*, **1995**, *689*, 31–38 [LC-MS]

Bareggi, S.R.; Cinque, P.; Mazzei, M.; D'Arminio, A.; Ruggieri, A.; Pirola, R.; Nicolin, A.; Lazzarin, A. Pharmacokinetics of zidovudine in HIV-positive patients with liver disease. *J.Clin.Pharmacol.*, **1994**, *34*, 782–786 [plasma; extracted metabolites; LOD 10 ng/mL]

Nebinger, P.; Koel, M. Determination of serum zidovudine by ultrafiltration and high-performance liquid chromatography. *J.Pharm.Biomed.Anal.*, **1994**, *12*, 141–143 [ultrafiltrate; LOD 50 ng/mL; adenine (IS)]

Good, S.S.; Reynolds, D.J.; de Miranda, P. Simultaneous quantification of zidovudine and its glucuronide in serum by high-performance liquid chromatography. *J.Chromatogr.*, **1988**, *431*, 123–133

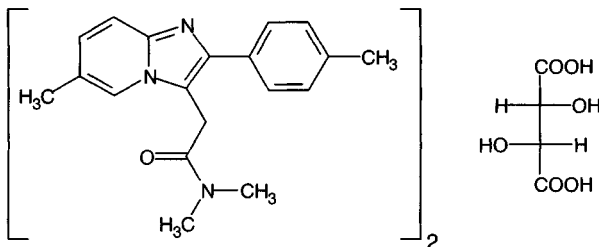
Unadkat, J.D.; Crosby, S.S.; Wang, J.P.; Hertel, C.C. Simple and rapid high-performance liquid chromatographic assay for zidovudine (azidothymidine) in plasma and urine. *J.Chromatogr.*, **1988**, *430*, 420–423

Zolpidem

Molecular formula: C₁₉H₂₁N₃O

Molecular weight: 307.4

CAS Registry No.: 82626-48-0 (zolpidem), 99294-93-6 (zolpidem (+)-tartrate (2:1))



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)) KH₂PO₄ 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 239

CHROMATOGRAM

Retention time: 4.66

Limit of detection: <120 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, acepromazine, aceprometazine, acetaminophen, aconitine, albuterol, alimemazine, alpidem, alprazolam, alprenolol, amisulpride, amitriptyline, amoxapine, aspirin, astemizole, atenolol, benazepril, benzocaine, benzoylcegonine, bepridil, betaxolol, bisoprolol, bromazepam, brompheniramine, bumadizone, bupivacaine, buprenorphine, buspirone, caffeine, carbamazepine, carbinoxamine, carpiramine, carteolol, cetirizine, chlorambucil, chlordiazepoxide, chlormezanone, chlorphenacinone, chlorpheniramine, chlorpromazine, chlorpropamide, cibenzoline, clemastine, clobazam, clomipramine, clonazepam, clonidine, clorazepate, clozapine, codeine, colchicine, cyamemazine, cyclizine, cycloguanil, cyproheptadine, cytarabine, dacarbazine, daunorubicin, debrisoquine, demexiptiline, desipramine, dextromethorphan, dextromoramide, dextropropoxyphene, diazepam, diazoxide, diclofenac, dihydralazine, diltiazem, diphenhydramine, dipyridamole, disopyramide, dosulepine, doxepin, ephedrine, estazolam, etodolac, fenfluramine, fenpropfen, fentiazac, flecainide, floctafenine, flumazenil, flunitrazepam, fluoxetine, fluphenazine, flurbiprofen, fluvoxamine, glibenclamide, glibornuride, glipizide, glutethimide, haloperidol, histapyrrodine, hydroxyzine, ibuprofen, imipramine, indomethacin, iproniazid, ketamine, levomepromazine, lidoflazine, lisinopril, loperamide, loprazolam, lorata-

dine, lorazepam, loxapine, maprotiline, medazepam, medifoxamine, mefenamic acid, mephenidramine, mefloquine, melphalan, mephesisin, mephentermine, metapramine, metformin, methadone, methaqualone, methocarbamol, methotrexate, metipranolol, metoclopramide, metoprolol, mianserine, midazolam, minoxidil, moperone, morphine, nadolol, nalbuphine, nalorphine, naloxone, naltrexone, naproxen, nialamide, nicardipine, nifedipine, niflumic acid, nimodipine, nitrazepam, nitrendipine, nizatidine, nortriptyline, omeprazole, opipramol, oxazepam, oxprenolol, penbutolol, penfluridol, pentazocine, phenacyclidine, phenobarbital, phenol, phenylbutazone, pimozide, pindolol, pipamperone, piroxicam, prazepam, prazosin, prilocaine, procainamide, procarbazine, proguanil, promethazine, propafenone, propranolol, protriptyline, pyrimethamine, quinidine, quinine, quinupramine, ramipril, ranitidine, reserpine, ritodrine, sotalol, strychnine, sulfinpyrazole, sulindac, sulpride, sultopride, suriclone, tenoxicam, terbutaline, terfenadine, tetracaine, tetrazepam, thiopental, thioproperazine, thioridazine, tianeptine, tiapride, tiaprofenic acid, tiocloamarol, tofisopam, tolbutamide, toloxatone, trazodone, triazolam, trifluoperazine, trifluoperidol, trimipramine, triprolidine, tropatenine, verapamil, vinblastine, vincristine, warfarin, zopiclone, zorubicine

Interfering: acenocoumarol, ajmaline, alminoprofen, amodiaquine, benperidol, celiprolol, chloroquine, cicletanine, cocaine, doxylamine, droperidol, hydroxychloroquine, ketoprofen, labetalol, lidocaine, meperidine, mepivacaine, mexiletine, moclobemide, nomifensine, secobarbital, temazepam, ticlopidine, timolol, viloxazine, vindesine, yohimbine

KEY WORDS

whole blood; plasma

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: 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 μ L mobile phase, inject a 50 μ L aliquot. (Buffer was saturated ammonium chloride, diluted 25% with water, adjusted to pH 9.5 with 25% diluted ammonia solution.)

HPLC VARIABLES

Column: 300 \times 3.9 μ m 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 207

CHROMATOGRAM

Retention time: 4.66

Limit of detection: 23.1 ng/mL

OTHER SUBSTANCES

Extracted: alpidem, suriclone, zopiclone

Simultaneous: nimodipine, p-nitrophenol, pyrimethamine, sultopride, tiaprofenic acid, vincristine

Interfering: ketotifen

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 Serum or plasma + 1 mL saturated Na_2HPO_4 + 100 μL 10 $\mu\text{g}/\text{mL}$ IS + 5 mL diisopropyl ether:isopropanol 95:5 (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 5 min, centrifuge. Remove the organic layer and evaporate it to dryness at 35°, reconstitute the residue in 50 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 100 \times 3 Chrompack CP spher C8**Mobile phase:** MeCN:50 mM NaH_2PO_4 70:30 adjusted to pH 2.2 with 85% orthophosphoric acid**Flow rate:** 0.8**Injection volume:** 20**Detector:** UV 210

CHROMATOGRAM**Retention time:** 2.6**Internal standard:** N-6-dimethyl-2-(4-methylphenyl)-N-propylimidazo[1,2- α]pyridine-3-acetamide methanesulfonate (Synthelabo France) (6.4)

OTHER SUBSTANCES**Extracted:** flumazenil, prothipendyl

KEY WORDS

serum; plasma; pharmacokinetics

REFERENCE

Debailleul, G.; Khalil, F.A.; Lheureux, P. HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication. *J.Anal.Toxicol.*, **1991**, *15*, 35–37

SAMPLE**Matrix:** blood, urine

Sample preparation: Dilute urine 10-fold with water. 1 mL Plasma or diluted urine + 20 μL 1.5 $\mu\text{g}/\text{mL}$ IS in MeOH, vortex, centrifuge at 11000 g for 3 min, inject 100 (plasma) or 50 (diluted urine) μL supernatant onto column A and elute to waste with mobile phase A, after 2 min elute the contents of column A onto column B with mobile phase B, after 1.5 min remove column B from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Backflush column A with MeCN:water 50:50 and MeOH:water 50:50 then forward flush with water.

HPLC VARIABLES**Column:** A 75 \times 2.1 30-40 μm Perisorb C18 (Merck); B 20 \times 4.6 40 μm Pelliguard (Supelco) + 150 \times 4.6 5 μm Supelcosil LC 18-DB**Mobile phase:** A water; B MeCN:MeOH:buffer 60:0.75:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.0 with 1 M KOH.)**Flow rate:** A 2; B 1

Injection volume: 80-200
Detector: F ex 254 em 390

CHROMATOGRAM

Retention time: 15.8
Internal standard: N-6-dimethyl-2-(4-methylphenyl)-N-propylimidazo[1,2- α]pyridine-3-acetamide (SL 83.0725, Synthélabo, France) (13.1)
Limit of detection: 0.2 ng/mL (plasma); 1 ng/mL (urine)
Limit of quantitation: 1 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: trazodone
Noninterfering: diazepam, flunitrazepam, lorazepam, nordiazepam, oxazepam, triazolam

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Ascalone, V.; Flaminio, L.; Guinebault, P.; Thénot, J.P.; Morselli, P.L. Determination of zolpidem, a new sleep-inducing agent, and its metabolites in biological fluids: pharmacokinetics, drug metabolism and overdosing investigations in humans. *J.Chromatogr.*, **1992**, *581*, 237-250

SAMPLE

Matrix: blood, gastric contents, tissue, urine
Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μ L 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 μ L 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μ L 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 \times 4 30 μ m LiChrocart Aluspher RP-select B (Merck)
Column: 125 \times 4 5 μ m 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; UV 254

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, pindolol
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, tripeleppamine, 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: hepatocyte suspensions

Sample preparation: 150 μ L Hepatocyte suspension + 150 μ L MeCN, centrifuge at 5000 g, add 250 ng IS, inject a 150 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 125 \times 4.5 μ m Lichrosphere 60

Mobile phase: MeCN:MeOH:20 mM pH 4.5 potassium phosphate buffer 4:30:66

Flow rate: 0.9

Injection volume: 150

Detector: F ex 254 em 390

CHROMATOGRAM

Retention time: 20.84

Internal standard: SL 870105 (Synthelabo-Recherche, Paris) (22.56)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver

REFERENCE

Pichard, L.; Gillet, G.; Bonfils, C.; Domergue, J.; Thénot, J.-P.; Maurel, P. Oxidative metabolism of zolpidem by human liver cytochrome P450S. *Drug Metab. Dispos.*, **1995**, *23*, 1253–1262

SAMPLE

Matrix: microsomal incubations

Sample preparation: 500 μ L Microsomal incubation + 500 μ L MeCN, centrifuge at 5000 g, add IS to the supernatant at a concentration of 6.7 μ g/mL, inject a 150 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 μ m Interchrom C6 (Interchim, Montluçon, France)

Mobile phase: MeCN:25 mM pH 6.0 potassium phosphate buffer 30:65

Flow rate: 1

Injection volume: 150

Detector: F ex 254 em 390

CHROMATOGRAM

Retention time: 18.90

Internal standard: N-dealkylated alpidem (13.20)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver

REFERENCE

Pichard, L.; Gillet, G.; Bonfils, C.; Domergue, J.; Thénot, J.-P.; Maurel, P. Oxidative metabolism of zolpidem by human liver cytochrome P450S. *Drug Metab. Dispos.*, **1995**, *23*, 1253-1262

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 KH₂PO₄ and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 203; UV 233; UV 292

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, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, 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

ANNOTATED BIBLIOGRAPHY

Vajta, S.; De Maack, F.; Devant, G.; Lesieur, M. Thermospray liquid chromatography tandem mass spectrometry: application to the elucidation of zolpidem metabolism. *Biomed. Environ. Mass. Spectrom.*, **1988**, *15*, 223-228

NAME INDEX

NAME

2-G
27-400
38489
4311/b Ciba
44089
640/359
66873
A 43818
A 56268
Aarane
Aaarre
AB08
Abacin
Abacin
Abbocillin V
Abboticine (erythromycin stearate)
Abomecatin
Abbott-43818
Abbott 44090
Abbott 45975
Abbott-50711
Abbott-56268
Abensanil
Aberel (tretinoin)
Abricycline
Acamol
Accupril
Accuprin
Accupro
Accurbron
Accuretic
Accuretic
Accutane (isotretinoin)
Acediur
Acenalin
Acenterine

MONOGRAPH

Guaifenesin
Cyclosporine
Nortriptyline
Methylphenidate
Valproic Acid
Cefuroxime
Cephalexin
Leuprolide
Clarithromycin
Cromolyn
Cromolyn
Doxycycline
Sulfamethoxazole
Trimethoprim
Penicillin V
Erythromycin
Erythromycin
Leuprolide
Valproic Acid
Terazosin
Valproic Acid
Clarithromycin
Acetaminophen
Retinoic Acid
Tetracycline
Acetaminophen
Quinapril
Quinapril
Quinapril
Theophylline
Hydrochlorothiazide
Quinapril
Retinoic Acid
Captopril
Cisapride
Aspirin

NAME	MONOGRAPH
Aceplus	Captopril
Acepress	Captopril
Acepril	Captopril
Acequin	Quinapril
Acerbon	Lisinopril
Acesistem	Enalapril
Acetalgin	Acetaminophen
<i>p</i> -acetamidophenol	Acetaminophen
Acetexa	Nortriptyline
Aceticyl	Aspirin
Acetillum Acidulatum	Aspirin
Acetonyl	Aspirin
3-(α -acetonylbenzyl)-4-hydroxycoumarin	Warfarin
Acetophen	Aspirin
Acetosal	Aspirin
Acetosalic acid	Aspirin
Acetosalin	Aspirin
2-acetoxybenzoic acid	Aspirin
17 α -acetoxy-6 α -methylprogesterone	Medroxyprogesterone Acetate
Acet-Theocin Sodium	Theophylline
cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1 <i>H</i> -imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine	Ketoconazole
<i>N</i> -acetyl- <i>p</i> -aminophenol	Acetaminophen
Acetyl-SAL	Aspirin
<i>p</i> -acetylamino-phenol	Acetaminophen
Acetylin	Aspirin
(2 <i>S</i> -cis)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5 <i>H</i>)-one	Diltiazem
(6 α)-17-(acetyloxy)-6 α -methylpregn-4-ene-3,20-dione	Medroxyprogesterone Acetate
2-(acetyloxy)benzoic acid	Aspirin
[6 <i>R</i> -[6 α ,7 β (<i>Z</i>)]]-3-[(acetyloxy)methyl]-7-[[2-amino-4-thiazolyl(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefotaxime
acetylsalicylic acid	Aspirin
Acezide	Captopril
Achro	Tetracycline
Achromycin V	Tetracycline
Achromycin	Tetracycline
Acibilin	Cimetidine
Acicloftal	Acyclovir
aciclovir	Acyclovir
acidum acetylsalicylicum	Aspirin
Acillin	Ampicillin
Aciloc	Cimetidine
Acimetten	Aspirin
Acinil	Cimetidine
Acipen-V	Penicillin V

NAME

Aclovir
 Acrofolin (estradiol propionate)
 Acticort
 Actifed Plus
 Actifed
 Actifed-C
 Actilin
 Actilyse
 Actimmune (Cys-Tyr-Cys-interferon-gamma)
 Actiplas
 Activase
 Actocortin (hydrocortisone sodium phosphate)
 Acuitel
 Acular
 acycloguanosine
 Acylpyrin
 Adalat
 Adalate
 Adapress
 Adcortyl (triamcinolone acetonide)
 Adcortyl-A (triamcinolone acetonide)
 ademin
 ademine
 adenoypophyseal growth hormone
 Adepril
 Adesitrin
 Adiab
 adiphenine
 Adisné
 Adizem
 Adobacillin
 Adofen
 Adoisine
 Adran
 Advantan (methylprednisolone aceponate)
 Advil
 Aerobec
 Aerobin
 Aerolate
 Aerolin
 Aeroseb-HC
 Aerosporin
 AF-2071 (hydrocortisone bendazac)
 Afonilum
 Agestal
 Agofollin (estradiol 3-benzoate)
 Agolanid
 Agram
 Agromicina
 AH 3365
 AH 19065

MONOGRAPH

Acyclovir
 Estradiol
 Hydrocortisone
 Acetaminophen
 Codeine
 Guaifenesin
 Neomycin
 Alteplase
 Interferon
 Alteplase
 Alteplase
 Hydrocortisone
 Quinapril
 Ketorolac
 Acyclovir
 Aspirin
 Nifedipine
 Nifedipine
 Nifedipine
 Nifedipine
 Triamcinolone
 Triamcinolone
 Triamterene
 Triamterene
 Somatropin
 Amitriptyline
 Nitroglycerin
 Glyburide
 Adiphenine
 Theophylline
 Diltiazem
 Ampicillin
 Fluoxetine
 Warfarin
 Ibuprofen
 Methylprednisolone
 Ibuprofen
 Beclomethasone Dipropionate
 Theophylline
 Theophylline
 Albuterol
 Hydrocortisone
 Polymyxin
 Hydrocortisone
 Theophylline
 Medroxyprogesterone Acetate
 Estradiol
 Digoxin
 Amoxicillin
 Tetracycline
 Albuterol
 Ranitidine

NAME	MONOGRAPH
A-hydro Cort (hydrocortisone 21-sodium succinate)	Hydrocortisone
Airol (tretinoin)	Retinoic Acid
Aisemide	Furosemide
Ak-Mycin	Erythromycin
Aknin	Erythromycin
Aknoten (tretinoin)	Retinoic Acid
Akrofolin (estradiol propionate)	Estradiol
Ala Tet	Tetracycline
Ala-Cort	Hydrocortisone
Alapril	Lisinopril
Albipen	Ampicillin
Aldaban	Terfenadine
Aldactazide	Hydrochlorothiazide
Aldecin	Beclomethasone Dipropionate
Aldipin	Nifedipine
Aldoril	Hydrochlorothiazide
Alepsin	Phenytoin
Alercrom	Cromolyn
Alerion	Cromolyn
Aleve	Naproxen
Alfacet	Cefaclor
Alfadat	Nifedipine
Alfadil	Doxazosin
Alfamox	Amoxicillin
Alfason (hydrocortisone 17-butyrate)	Hydrocortisone
Alfaspoven	Cephalexin
Alfatil	Cefaclor
Alferon N (Alfa-n3)	Interferon
Alferon N (Alfa-n3)	Interferon
Alferon Gel (Alfa-2b)	Interferon
Algafan	Propoxyphene
Alimix	Cisapride
alisobumal	Butalbital
Allegron	Nortriptyline
Allerest Sinus Pain	Acetaminophen
Allergocrom	Cromolyn
Allerplus	Terfenadine
Allvoran	Diclofenac
allylbarbital	Butalbital
5-allyl-5-isobutylbarbituric acid	Butalbital
5-allyl-5-(2-methylpropyl)barbituric acid	Butalbital
Almazine	Lorazepam
Almodan	Amoxicillin
Alopresin	Captopril
Alpen	Ampicillin
Alpen-N	Ampicillin
Alphaderm	Hydrocortisone
Alphatrex (betamethasone 17,21-dipropionate)	Betamethasone
Alpiny	Acetaminophen
Alplax	Alprazolam
Alrheumat	Ketoprofen

NAME	MONOGRAPH
Alrheumon	Ketoprofen
Altace	Ramipril
Altiazem	Diltiazem
Altilev	Nortriptyline
Alupram	Diazepam
Aluzine	Furosemide
Alvo	Oxaprozin
Amadil	Acetaminophen
Ambien	Zolpidem
Amblosin	Ampicillin
Ambracyn	Tetracycline
Ambramicina	Tetracycline
Ambramycin	Tetracycline
Amcap	Ampicillin
Amcill	Ampicillin
Amcill-S	Ampicillin
Amen	Medroxyprogesterone Acetate
Amenyl	Ethinyl Estradiol
Amfamox	Famotidine
Amfipen	Ampicillin
Amibufen	Ibuprofen
Amineurin	Amtriptyline
4-amino-2-[4-(1,4-benzodioxan-2-carbonyl) piperazin-1-yl]-6,7-dimethoxyquinazoline	Doxazosin
D-(—)- α -aminobenzylpenicillin	Ampicillin
[6 <i>R</i> -[6 α ,7 β (<i>Z</i>)]]-3-[[[(aminocarbonyl)oxy]methyl]- 7-[[2-furanyl(methoxyimino)acetyl]amino]-8- oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2- carboxylic acid	Cefuroxime
[<i>R</i> -[<i>R</i> *, <i>S</i> *(<i>Z</i>)]]-7-[(2-amino-2-carboxyethyl)thio]- 2-[[[2,2-dimethylcyclopropyl]carbonyl]amino]- 2-heptanoic acid	Cilastatin
<i>cis</i> -4-amino-5-chloro- <i>N</i> -[1-[3-(<i>p</i> - fluorophenoxy)propyl]-3-methoxy-4- piperidinyl]- <i>o</i> -anisamide	Cisapride
<i>cis</i> -4-amino-5-chloro- <i>N</i> -[1-[3-(4- fluorophenoxy)propyl]-3-methoxy-4- piperidinyl]-2-methoxybenzamide	Cisapride
[1-amino-3-[[[2-[(diaminomethylene)amino]-4- thiazolyl]methyl] thio]propylidene]sulfamide	Famotidine
2-amino-1,9-dihydro-9-[(2- hydroxyethoxy)methyl]-6 <i>H</i> -purin-6-one	Acyclovir
1-(4-amino-6,7-dimethoxy-2-quinazoliny)-4-[(2,3- dihydro-1,4-benzodioxin-2- yl)carbonyl]piperazine	Doxazosin
1-(4-amino-6,7-dimethoxy-2-quinazoliny)-4- [(tetrahydro-2-furanyl)carbonyl]piperazine	Terazosin
2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)- 1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester	Amlodipine

NAME	MONOGRAPH
(±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine	Amlodipine
(<i>R</i> *, <i>S</i> *)-α-(±)-α-(1-aminoethyl)benzene-methanol hydrochloride	Phenylpropanolamine
α-(1-aminoethyl)benzyl alcohol hydrochloride	Phenylpropanolamine
α-amino- <i>p</i> -hydroxybenzylpenicillin	Amoxicillin
7-[D-(—)-α-amino-α-(4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid	Cefadroxil
(6 <i>R</i> , 7 <i>R</i>)-7-[(<i>R</i>)-2-amino-2-(<i>p</i> -hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefprozil
6-[D-(—)-α-amino- <i>p</i> -hydroxyphenylacetamido]penicillanic acid	Amoxicillin
[2 <i>S</i> -[2α,5α,6β(<i>S</i> *)]]-6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	Amoxicillin
7-[[amino-4-(hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefadroxil
[6 <i>R</i> -[6α,7β(<i>R</i> *)]]-7-[[amino(4-hydroxyphenyl)acetyl]amino]-8-oxo-3-(1-propenyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefprozil
3-[[[2-(aminoiminomethyl)amino]-4-thiazolyl]methyl]thio]- <i>N</i> -(aminosulfonyl)propanimidamide	Famotidine
4-amino- <i>N</i> -(5-methyl-3-isoxazolyl)benzensulfonamide	Sulfamethoxazole
7-(D-2-amino-2-phenylacetamido)-3-chloro-3-cephem-4-carboxylic acid	Cefaclor
(6 <i>R</i> , 7 <i>S</i>)-7-[(<i>R</i>)-2-amino-2-phenylacetamido]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Loracarbef
(6 <i>R</i> , 7 <i>R</i>)-7-[(<i>R</i>)-2-amino-2-phenylacetamido]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefaclor
7-(D-α-aminophenylacetamido)desacetoxycephalosporanic acid	Cephalexin
7-(D-2-amino-2-phenylacetamido)-3-methyl-delta ³ -cephem-4-carboxylic acid	Cephalexin
6-[D-(—)-α-aminophenylacetamido]penicillanic acid	Ampicillin
[6 <i>R</i> -[6α,7β(<i>R</i> *)]]-7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Loracarbef

NAME	MONOGRAPH
7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefaclor
[6 <i>R</i> -[6 α ,7 β (<i>R</i> *)]-7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefaclor
6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	Ampicillin
7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cephalexin
2-amino-1-phenyl-1-propanol hydrochloride	Phenylpropanolamine
3-(<i>p</i> -aminophenylsulfonamido)-5-methylisoxazole	Sulfamethoxazole
5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid	Furosemide
3-(aminosulfonyl)-4-chloro- <i>N</i> -(2,3-dihydro-2-methyl-1 <i>H</i> -indol-1-yl)benzamide	Indapamide
7-[2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid	Cefixime
[6 <i>R</i> -[6 α ,7 β (<i>Z</i>)]]-7-[(2-amino-4-thiazolyl)(carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefixime
[6 <i>R</i> -[6 α ,7 β (<i>Z</i>)]]-1-[[7-[(2-amino-4-thiazolyl)(1-carboxy-1-methylethoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-yl]methyl] pyridinium hydroxide, inner salt	Ceftazidime
1-[[6 <i>R</i> ,7 <i>R</i>]-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-yl]methyl] pyridinium hydroxide, inner salt	Ceftazidime
7 ² -(<i>Z</i>)-[<i>O</i> -(1-carboxy-1-methylethyl)oxime]	
(6 <i>R</i> ,7 <i>R</i>)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-[[[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo- <i>s</i> -triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Ceftriaxone
7 ² -(<i>Z</i>)-(O-methyloxime)	
(6 <i>R</i> ,7 <i>R</i>)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefotaxime
7 ² -(<i>Z</i>)-(O-methyloxime) acetate	
7-[2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]cephalosporanic acid	Cefotaxime
[6 <i>R</i> -[6 α ,7 β (<i>Z</i>)]]-7-[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Ceftriaxone

NAME

Anaspaz
 Anceron
 Anco
 Ancortone
 Andion
 Andozac
 Androgyn L.A. (estradiol 17-valerate)
 Anemolin
 Anergan 25
 Anergan 50
 Anexsia
 Anexasia
 Anflagen
 Anflam
 Angibid
 Anginine
 Anginyl
 Angiolingual
 Angizem
 Angorin
 Anhiba
 anhydrohydroxynorprogesterone
 Anifed
 Anisolin
 Anodynos-DHC
 Anovlar (norethindrone acetate)
 Ansial
 Ansiced
 Antacal
 Antalvic
 Antepsin
 anterior pituitary growth hormone
 Antibiocin
 antibiotique EF 185
 anti-inflammatory hormone
 Antirobe
 Antisacer
 Antra
 Antuitrin-Growth
 Anusol-HC (hydrocortisone acetate)
 Apamide
 APAP
 Apaurin
 Apesan
 Apopen
 Apo-Sulfatrim
 Apo-Sulfatrim
 Apozepam
 Apranax
 Apresazide
 Aprical
 Apsifen

MONOGRAPH

Atropine
 Beclomethasone Dipropionate
 Ibuprofen
 Prednisone
 Beclomethasone Dipropionate
 Finasteride
 Estradiol
 Amoxicillin
 Promethazine
 Promethazine
 Acetaminophen
 Hydrocodone
 Ibuprofen
 Hydrocortisone
 Nitroglycerin
 Nitroglycerin
 Diltiazem
 Nitroglycerin
 Diltiazem
 Nitroglycerin
 Acetaminophen
 Norethindrone
 Nifedipine
 Diazepam
 Hydrocodone
 Norethindrone
 Buspirone
 Buspirone
 Amlodipine
 Propoxyphene
 Sucralfate
 Somatropin
 Penicillin V
 Neomycin
 Hydrocortisone
 Clindamycin
 Phenytoin
 Omeprazole
 Somatropin
 Hydrocortisone
 Acetaminophen
 Acetaminophen
 Diazepam
 Carisoprodol
 Penicillin V
 Sulfamethoxazole
 Trimethoprim
 Diazepam
 Naproxen
 Hydrochlorothiazide
 Nifedipine
 Ibuprofen

NAME

Apsin VK
 Aquadiol
 Aquanil
 Aquarius
 Aquo-Trinitrosan
 Arcacil
 Arcasin
 Ardine
 Arial
 Aristocort Forte Parenteral (triamcinolone diacetate)
 Aristocort
 Aristocort Syrup (triamcinolone diacetate)
 Aristoderm (triamcinolone acetone)
 Aristosol (triamcinolone acetone 21-disodium phosphate)
 Aristospan (triamcinolone hexacetone)
 A.R.M.
 Armophylline
 Arpamyl
 Arpimycin (erythromycin ethylsuccinate)
 Arthaxan
 Arthritis
 Artisone-Wyeth
 Artomycin
 Artrene
 Artril 300
 Artrosilene
 Artroxicam
 Arusal
 A.S.A.
 A.S.A.
 Asatard
 Ascriptin
 Asellacrin
 Asmaven
 Aspenil
 Aspirin-Free Anacin
 Aspro
 Assaren
 Astemisan
 Asteric
 Ateben
 AteHexal
 Atem
 Atenol
 Atensine
 Athrombin-K
 Atilen
 Ativan
 Atosil
 AtroDote

MONOGRAPH

Penicillin V
 Estradiol
 Timolol
 Hydrochlorothiazide
 Nitroglycerin
 Penicillin V
 Penicillin V
 Amoxicillin
 Salmeterol
 Triamcinolone
 Triamcinolone
 Triamcinolone
 Triamcinolone
 Triamcinolone
 Phenylpropanolamine
 Theophylline
 Verapamil
 Erythromycin
 Nabumetone
 Aspirin
 Methylprednisolone
 Tetracycline
 Ibuprofen
 Ibuprofen
 Ketoprofen
 Piroxicam
 Carisoprodol
 Aspirin
 Codeine
 Aspirin
 Aspirin
 Somatropin
 Albuterol
 Amoxicillin
 Acetaminophen
 Aspirin
 Diclofenac
 Astemizole
 Aspirin
 Nortriptyline
 Atenolol
 Ipratropium Bromide
 Atenolol
 Diazepam
 Warfarin
 Diazepam
 Lorazepam
 Promethazine
 Atropine

NAME

Atrophate
 Atropisol
 atroscine
 Atrosed
 Atrovent
 A/T/S
 Atumin
 Augmentin
 Augmentin
 Ausocef
 Austrapen
 Austyn
 Avantyl
 Aventyl
 Aventyl Hydrochloride
 Avomine
 AX 250
 Axer Alfa
 Axid
 Axoren
 Axoril (cefuroxime axetil)
 Ay 6108
 AY24236
 Aygestin (norethindrone acetate)
 Azamune
 Azanin
 Azantac
 azido-3'-deoxythymidine 3'-
 azidothymidine
 Azitrocin
 Azmacort (triamcinolone acetonide)
 Azo-Gantanol
 Azoran
 azothioprine
 Azotrex
 AZT
 Aztec
 Azudoxat
 Azuglucon
 Azupentat
 Bactoderm
 Bactramin
 Bactramin
 Bactrim
 Bactrim
 Bactroban
 Bactromin
 Bactromin
 Bagren
 Bajaten
 Baktar
 Baktar

MONOGRAPH

Atropine
 Atropine
 Scopolamine
 Atropine
 Ipratropium Bromide
 Erythromycin
 Dicyclomine
 Amoxicillin
 Clavulanic Acid
 Cephalixin
 Ampicillin
 Theophylline
 Nortriptyline
 Nortriptyline
 Nortriptyline
 Promethazine
 Amoxicillin
 Naproxen
 Nizatidine
 Buspirone
 Cefuroxime
 Ampicillin
 Etodolac
 Norethindrone
 Azathioprine
 Azathioprine
 Ranitidine
 Zidovudine
 Zidovudine
 Azithromycin
 Triamcinolone
 Sulfamethoxazole
 Azathioprine
 Azathioprine
 Tetracycline
 Zidovudine
 Zidovudine
 Doxycycline
 Glyburide
 Pentoxifylline
 Mupirocin
 Sulfamethoxazole
 Trimethoprim
 Trimethoprim
 Sulfamethoxazole
 Mupirocin
 Trimethoprim
 Sulfamethoxazole
 Bromocriptine
 Indapamide
 Sulfamethoxazole
 Trimethoprim

NAME

Balmox
 Banesin
 Bantogen
 Barbidonna
 Barbidonna
 Bassado
 Bastiverit
 Batrilix
 Baxan
 Baxo
 BAY a 1040
 BAY b 5097
 Baycip
 Bayer
 Bay o 9867
 Bay q 3939
 BBS-1067
 Be-100 (ibuprofen piconol)
 Bebate (betamethasone 17-benzoate)
 Beben (betamethasone 17-benzoate)
 Beclacin
 Becloforte
 Beclomet
 beclomethasone
 Beclorhinol
 Becloval
 Beclovent
 Becodisks
 Beconase
 Beconasol
 Becort
 Becotide
 Bedermin (betamethasone 17-valerate)
 Benisone (betamethasone 17-benzoate)
 Beepen-VK
 Bekadid
 Beloc
 Ben-u-ron
 Benacine
 Benacol
 benazepril
 bendacort (hydrocortisone bendazac)
 Benfofen
 Benovocylin (estradiol 3-benzoate)
 Bentelan (betamethasone 21-phosphate disodium salt)
 Bentomine
 Bently Hydrochloride
 Bentlyl Hydrochloride
 Benzamycin
 Benzhormovarine (estradiol 3-benzoate)
 Benzo-Gynöestryl (estradiol 3-benzoate)

MONOGRAPH

Nabumetone
 Acetaminophen
 Penicillin V
 Atropine
 Scopolamine
 Doxycycline
 Glyburide
 Indapamide
 Cefadroxil
 Piroxicam
 Nifedipine
 Clotrimazole
 Ciprofloxacin
 Aspirin
 Ciprofloxacin
 Ciprofloxacin
 Cefprozil
 Ibuprofen
 Betamethasone
 Betamethasone
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Betamethasone
 Beclomethasone Dipropionate
 Betamethasone
 Betamethasone
 Penicillin V
 Hydrocodone
 Metoprolol
 Acetaminophen
 Scopolamine
 Dicyclomine
 Benazepril
 Hydrocortisone
 Diclofenac
 Estradiol
 Betamethasone

 Dicyclomine
 Dicyclomine
 Dicyclomine
 Erythromycin
 Estradiol
 Estradiol

NAME	MONOGRAPH
Benzoestrofol (estradiol 3-benzoate)	Estradiol
Benzofoline (estradiol 3-benzoate)	Estradiol
[2aR-[2a α ,4 β ,4a β ,6 β ,9(α R*, β S*),11 α ,12 α ,12b α]]- β -(benzoylamino)- α -hydroxybenzene-propanoic acid 6,12b-bis(acetyloxy)-12-(benzoyloxy)- 2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro- 4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo- 7,11-methano-1 <i>H</i> -cyclodeca[3,4]benz[1,2- <i>b</i>]- oxet-9-yl ester	Paclitaxel
(\pm)-5-benzoyl-2,3-dihydro-1 <i>H</i> -pyrrolizine-1- carboxylic acid	Ketorolac
5-benzoyl-1,2-dihydro-3 <i>H</i> -pyrrolo[1,2- <i>a</i>]pyrrole- 1-carboxylic acid	Ketorolac
<i>m</i> -benzoylhydratropic acid	Ketoprofen
3-benzoyl- α -methylbenzeneacetic acid	Ketoprofen
2-(3-benzoylphenyl)propionic acid	Ketoprofen
Benztrone (estradiol 3-benzoate)	Estradiol
Berkatens	Verapamil
Berkfuran	Nitrofurantoin
Berkfurin	Nitrofurantoin
Berofor Alpha 2 (Alfa-2c)	Interferon
Beromycin	Penicillin V
Beromycin 400	Penicillin V
Beronald	Furosemide
Bespar	Buspirone
Betabactyl	Clavulanic Acid
beta-Corlan	Betamethasone
betadexamethasone	Betamethasone
Betadival	Betamethasone
Betafluorene (betamethasone 21-acetate)	Betamethasone
Betaloc	Metoprolol
Betamazee	Sulbactam
Betamox	Amoxicillin
Betapen VK	Penicillin V
Betaseron (Beta-1b)	Interferon
Betasolon	Betamethasone
Betasone (betamethasone 17,21-dipro- pionate)	Betamethasone
Betatrex (betamethasone 17-valerate)	Betamethasone
BetaVal (betamethasone 17-valerate)	Betamethasone
Betavet Soluspan	Betamethasone
Bethacil	Sulbactam
Betim	Timolol
Betnelan	Betamethasone
Betnesol (betamethasone 21-phosphate disodium salt)	Betamethasone
Betnesol-V (betamethasone 17-valerate)	Betamethasone
Betneval (betamethasone 17-valerate)	Betamethasone
Betnovate (betamethasone 17-valerate)	Betamethasone
Betsovet (betamethasone 21-adamantoate)	Betamethasone
Bextasol (betamethasone 17-valerate)	Betamethasone
Bialzepam	Diazepam

NAME	MONOGRAPH
Biaxin	Clarithromycin
Bicillin L-A	Penicillin V
Bickie-mol	Acetaminophen
Bicortone	Prednisone
[1,1'-bicyclohexyl]-1-carboxylic acid 2-(diethylaminoethyl) ester	Dicyclomine
Bidocef	Cefadroxil
Bilordyl	Theophylline
Binotal	Ampicillin
Binovum	Ethinyl Estradiol
Binovum	Norethindrone
Bio-Tetra	Tetracycline
Bio-Tropin	Somatropin
Biociclin	Cefuroxime
Biocortar (hydrocortisone acetate)	Hydrocortisone
Biofurex	Cefuroxime
Biohulin	Insulin
Biomag	Cimetidine
Biophylline	Theophylline
Biosol	Neomycin
Bioxima	Cefuroxime
Biozolene	Fluconazole
Bi-Profenid	Ketoprofen
1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane	Cromolyn
bis(cyclohexyl)carboxylic acid diethylaminoethyl ester	Dicyclomine
2,6-bis(1-methylethyl)phenol	Propofol
Biston	Carbamazepine
Bitensil	Enalapril
Bitrop	Ipratropium Bromide
BL 191	Pentoxifylline
Blocadren	Timolol
BL-S 578	Cefadroxil
Bluton	Ibuprofen
BMY-28100	Cefprozil
BMY-28100-03-800	Cefprozil
BMY-28167	Cefprozil
Bonacid	Nifedipine
Bonapicillin	Ampicillin
Bonatranquan	Lorazepam
Bonyl	Naproxen
Bremil	Hydrochlorothiazide
Brevicon	Ethinyl Estradiol
Brevicon	Norethindrone
Brevinor	Ethinyl Estradiol
Brevinor	Norethindrone
Brexin (compound with β -cyclodextrin)	Piroxicam
Brexin EX	Guaifenesin
Briem	Benazepril
Bristaciclina	Tetracycline
Bristamox	Amoxicillin

NAME

Bristamycin (erythromycin stearate)
 Britacil
 Britiazem
 BRL 1341
 BRL 2333
 BRL-4910A
 BRL 14151
 BRL 14151K
 BRL 14777
 2-bromoergocryptine
 2-bromo- α -ergokryptin
 (5' α)-2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-
 (2-methylpropyl)ergotaman-3',6',18-trione
 Bronchoretard
 Broncovaleas
 Brondecon
 Bronkodyl
 Bronkotabs
 Brufanic
 Brufen
 Brufort
 Brumetidina
 Bruxicam
 Bruzem
 Buburone
 Buccalsone (hydrocortisone 21-sodium
 succinate)
 Bufferin
 Buspar
 Buspimen
 Buspinol
 buspirone
 Buspisal
 2-(*tert*-butylamino)-1-(4-hydroxy-3-
 hydroxymethylphenyl)ethanol
S-(—)-3-(3-*tert*-butylamino-2-hydroxypropoxy)-4-
 morpholino-1,2,5-thiadiazole
 α^1 -[(*tert*-butylamino)methyl]-4-hydroxy-*m*-
 xylene- α,α' -diol
 Butylenin
N-tert-butyl-3-oxo-4-aza-5 α -androst-1-ene-17 β -
 carboxamide
 1-(*p-tert*-butylphenyl)-4-[4'-(α -hydroxydi-
 phenylmethyl)-1'-piperidyl]butanol
 BW 56-72
 BW 57-322
 BW 72U
 BW 248U
 BW A509U
 Bykomycin
 Cabermox
 Calan

MONOGRAPH

Erythromycin
 Ampicillin
 Diltiazem
 Ampicillin
 Amoxicillin
 Mupirocin
 Clavulanic Acid
 Clavulanic Acid
 Nabumetone
 Bromocriptine
 Bromocriptine
 Bromocriptine
 Theophylline
 Albuterol
 Guaifenesin
 Theophylline
 Theophylline
 Ibuprofen
 Ibuprofen
 Ibuprofen
 Ibuprofen
 Cimetidine
 Piroxicam
 Diltiazem
 Ibuprofen
 Hydrocortisone
 Aspirin
 Buspirone
 Buspirone
 Buspirone
 Buspirone
 Buspirone
 Buspirone
 Albuterol
 Timolol
 Albuterol
 Ibuprofen
 Finasteride
 Terfenadine
 Trimethoprim
 Azathioprine
 Trimethoprim
 Acyclovir
 Zidovudine
 Neomycin
 Amoxicillin
 Verapamil

NAME	MONOGRAPH
Calcicard	Diltiazem
Calcipen	Penicillin V
Calcipen-V	Penicillin V
caldeCORT Spray	Hydrocortisone
CaldeCORT (hydrocortisone acetate)	Hydrocortisone
Calepsin	Carbamazepine
Caliment	Piroxicam
Calmaxid	Nizatidine
Calmipan	Guaifenesin
Calmodid	Hydrocodone
Calmpose	Diazepam
Calosen	Naproxen
Calpol	Acetaminophen
Camont	Nifedipine
Canesten	Clotrimazole
Canferon (Alfa-2a)	Interferon
Canifug	Clotrimazole
Capisten	Ketoprofen
Caplaril	Hydrochlorothiazide
Capoten	Captopril
Capozide	Captopril
Capozide	Hydrochlorothiazide
Caprin	Aspirin
Caprodat	Carisoprodol
Captea	Captopril
Captin	Acetaminophen
Captolane	Captopril
Captoril	Captopril
Carace	Lisinopril
Carafate	Sucralfate
carbacefaclor	Loracarbef
5-carbamoyl-5 <i>H</i> -dibenz[<i>b,f</i>]azepine	Carbamazepine
1- <i>p</i> -carbamoylmethylphenoxy-3-isopropylamino-2-propanol	Atenolol
(6 <i>R</i> ,7 <i>R</i>)-3-carbamoyloxymethyl-7-[2-(2-furyl)-2-(methoxyimino)acetamido]ceph-3-em-4-carboxylic acid	Cefuroxime
carbamoyloxymethyl-7-[2-(2-furyl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	Cefuroxime
Carbelan	Carbamazepine
<i>N</i> -(1 <i>S</i> -carboethoxy-3-phenylpropyl)- <i>S</i> -alanyl- <i>cis,endo</i> -2-azabicyclo[3.3.0]octane-3 <i>S</i> -carboxylic acid	Ramipril
1-[<i>N</i> -[(<i>S</i>)-1-carboxy-3-phenylpropyl]- <i>L</i> -alanyl]- <i>L</i> -proline 1'-ethyl ester	Enalapril
(2 <i>S</i> ,3 <i>aS</i> ,6 <i>aS</i>)-1-[(<i>S</i>)- <i>N</i> -[(<i>S</i>)-1-carboxy-3-phenylpropyl]alanyl]octahydrocyclopenta[<i>b</i>]pyrrole-2-carboxylic acid 1-ethyl ester	Ramipril

NAME	MONOGRAPH
(S)-2-[(S)-N-[(S)-1-carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid 1-ethyl ester	Quinapril
(3S)-3-[[[(1S)-1-carboxy-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, 3-ethyl ester	Benazepril
(S)-1-[N ² -(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline	Lisinopril
Carcinil	Leuprolide
Cardace	Ramipril
Cardamist	Nitroglycerin
Cardenalin	Doxazosin
Cardiagutt	Verapamil
Cardibeltin	Verapamil
Cardiem	Diltiazem
Cardiovet	Enalapril
Cardizem	Diltiazem
Cardular	Doxazosin
Cardura	Doxazosin
Carduran	Doxazosin
Cargosil	Acyclovir
Carisoma	Carisoprodol
carisoprodote	Carisoprodol
Carmol HC (hydrocortisone acetate)	Hydrocortisone
Cataflam	Diclofenac
CB-154	Bromocriptine
CB 311	Somatropin
CBCDA	Carboplatin
CCI 15641 (cefuroxime axetil)	Cefuroxime
CCI 23628 (cefuroxime pivoxetil)	Cefuroxime
Ceclor	Cefaclor
Cefa-Drops	Cefadroxil
Cefadros	Cephalexin
Cefa-Iskia	Cephalexin
Cefaloto	Cephalexin
Cefamar	Cefuroxime
Cefamox	Cefadroxil
Cefanex	Cephalexin
Cefaseptin	Cephalexin
cefatriaxone	Ceftriaxone
Cefibacter	Cephalexin
Cefixoral	Cefixime
Cefoprim	Cefuroxime
Ceforal	Cefadroxil
Cefossim	Cefuroxime
Cefotax	Cefotaxime
cefotaxime	Cefotaxime
Cefracycline	Tetracycline
Cefspan	Cefixime
Ceftim	Ceftazidime
Ceftin (cefuroxime axetil)	Cefuroxime
Cefumax	Cefuroxime

NAME	MONOGRAPH
Cefurax (cefuroxime axetil)	Cefuroxime
Cefurex	Cefuroxime
Cefurin	Cefuroxime
Cefzil	Cefprozil
Celestan (betamethasone 21-phosphate disodium salt)	Betamethasone
Celestan-V (betamethasone 17-valerate)	Betamethasone
Celeste	Triamcinolone
Celestene	Betamethasone
Celestoderm-V (betamethasone 17-valerate)	Betamethasone
Celestone	Betamethasone
Celestovet (betamethasone 21-acetate)	Betamethasone
Cenocort (triamcinolone diacetate)	Triamcinolone
Censpar	Buspirone
Centedrin	Methylphenidate
Cepazine (cefuroxime axetil)	Cefuroxime
Cephoral	Cefixime
Cephos	Cefadroxil
Ceporex	Cephalexin
Ceporexin	Cephalexin
Ceporexine	Cephalexin
Ceregulart	Diazepam
Cerepax	Temazepam
Cesplon	Captopril
Cetacort	Hydrocortisone
Cetadol	Acetaminophen
Cetampin	Ampicillin
Cétraphylline	Theophylline
Cetsim	Albuterol
Cex	Cephalexin
CG 315E	Tramadol
CGP 2175	Metoprolol
CGP 2175C	Metoprolol
CGP 2175E	Metoprolol
CGS-14824A	Benazepril
Chemcef	Cefotaxime
Chemiofuran	Nitrofurantoin
Chemotrim	Sulfamethoxazole
Chemotrim	Trimethoprim
Chibro-Proscar	Finasteride
Children's Tylenol	Acetaminophen
1-[[p-[2-(5-chloro- <i>o</i> -anisamido)ethyl]phenyl]sulfonyl]-3-cyclohexylurea	Glyburide
7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2 <i>H</i> -1,4-benzodiazepin-2-one	Lorazepam
5-chloro- <i>N</i> -[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-2-methoxybenzamide	Glyburide
7(<i>S</i>)-chloro-7-deoxylincomycin	Clindamycin
4-(8-chloro-5,6-dihydro-11 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i>]pyridin-11-ylidene)-1-piperidinecarboxylic acid ethyl ester	Loratadine

NAME	MONOGRAPH
6-chloro-3,4-dihydro-2 <i>H</i> -1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	Hydrochlorothiazide
7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2 <i>H</i> -1,4-benzodiazepin-2-one	Temazepam
7-chloro-1,3-dihydro-1-methyl-5-phenyl-2 <i>H</i> -1,4-benzodiazepin-2-one	Diazepam
6-chloro-3,4-dihydro-7-sulfamoyl-2 <i>H</i> -1,2,4-benzothiadiazine 1,1-dioxide	Hydrochlorothiazide
1-(<i>o</i> -chloro- α,α -diphenylbenzyl)imidazole	Clotrimazole
8-chloro-6-(2-fluorophenyl)-1-methyl-4 <i>H</i> -imidazo[1,5- <i>a</i>][1,4]benzodiazepine	Midazolam
4-chloro- <i>N</i> -4-furfuryl-5-sulfamoylanthranilic acid	Furosemide
4-chloro- <i>N</i> -4-(2-furylmethyl)-5-sulfamoylanthranilic acid	Furosemide
(11 β ,16 β)-9-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)pregna-1,4-diene-3,20-dione	Beclomethasone Dipropionate
4-chloro- <i>N</i> -(2-methyl-1-indoliny)-3-sulfamoylbenzamide	Indapamide
7-chloro-1-methyl-5-phenyl-3 <i>H</i> -1,4-benzodiazepin-2(1 <i>H</i>)-one	Diazepam
8-chloro-1-methyl-6-phenyl-4 <i>H</i> -[1,2,4]triazolo[4,3- <i>a</i>][1,4]benzodiazepine	Alprazolam
8-chloro-1-methyl-6-phenyl-4 <i>H</i> - <i>s</i> -triazolo[4,3- <i>a</i>][1,4]benzodiazepine	Alprazolam
8-chloro-11-(4-methyl-1-piperaziny)-5 <i>H</i> -dibenzo[<i>b,e</i>][1,4]diazepine	Clozapine
9 α -chloro-16 β -methylprednisolone 17,21-dipropionate	Beclomethasone Dipropionate
9 α -chloro-16 β -methyl-1,4-pregnadiene-11 α ,17 α ,21-triol-3,20 dione 17,21-dipropionate	Beclomethasone Dipropionate
Chloromycetin Hydrocortisone Ophthalmic (hydrortisone acetate)	Hydrocortisone
Chloromycin	Polymyxin
1-[α -(2-chlorophenyl)benzhydryl]imidazole	Clotrimazole
5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2 <i>H</i> -1,4-benzodiazepin-2-one	Clonazepam
1-[(<i>o</i> -chlorophenyl)diphenylmethyl]imidazole	Clotrimazole
1-[(2-chlorophenyl)diphenylmethyl]-1 <i>H</i> -imidazole	Clotrimazole
3-chloro-7- <i>D</i> -(2-phenylglycinamido)-3-cephem-4-carboxylic acid	Cefaclor
6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide	Hydrochlorothiazide
chlorosulthiadil	Hydrochlorothiazide
9-chloro-11 β ,17,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate	Beclomethasone Dipropionate
1-(<i>o</i> -chlorotriyl)imidazole	Clotrimazole
chlorsulfonamidodihydrobenzothiadiazine dioxide	Hydrochlorothiazide
Chlorzide	Hydrochlorothiazide

NAME	MONOGRAPH
Chronodalate	Nifedipine
CI-719	Gemfibrozil
CI-906	Quinapril
Ciba 4311b	Methylphenidate
Cibacen	Benazepril
Cibacène	Benazepril
Cibian (Alfa-2b)	Interferon
Ciblor	Clavulanic Acid
Cicladol (compound with β -cyclodextrin)	Piroxicam
ciclosporin	Cyclosporine
Cidrex	Hydrochlorothiazide
Ciflox	Ciprofloxacin
cilastatin	Cilastatin
Cilleral	Ampicillin
Ciloxan	Ciprofloxacin
Cimal	Cimetidine
Cimetag	Cimetidine
Cimetum	Cimetidine
cinnoxiam (piroxicam cinnamate)	Piroxicam
CINO-40 (triamcinolone diacetate)	Triamcinolone
Cinolone	Triamcinolone
Cipril	Cisapride
Ciprinol	Ciprofloxacin
Cipro	Ciprofloxacin
Cipro IV	Ciprofloxacin
Ciprobay	Ciprofloxacin
Ciproxan	Ciprofloxacin
Ciproxin	Ciprofloxacin
<i>cis</i> -estradiol	Estradiol
Citilat	Nifedipine
Citizem	Diltiazem
Citogel	Sucralfate
Citrullamon	Phenytoin
CL 19823	Triamcinolone
CL 34433 (triamcinolone hexacetonide)	Triamcinolone
CL 61965 (triamcinolone acetonide sodium phosphate)	Triamcinolone
CL 106359 (triamcinolone acetonide sodium phosphate)	Triamcinolone
CL 284635	Cefixime
Claforan	Cefotaxime
Clamoxyl	Amoxicillin
Clantin	Loratadine
Claradin	Aspirin
Colfarit	Aspirin
Claratal	Acetaminophen
Claritin	Loratadine
Clarityn	Loratadine
Clathromycin	Clarithromycin
clavulanic acid	Clavulanic Acid
Clear-Aid (hydrortisone acetate)	Hydrocortisone

NAME	MONOGRAPH
Cleiton (hydrocortisone 21-phosphate disodium salt)	Hydrocortisone
Clenil-A	Beclomethasone Dipropionate
Cleocin	Clindamycin
Cleocin HCl	Clindamycin
Cleocin Phosphate	Clindamycin
Cleocin T	Clindamycin
Clicil	Penicillin V
Climaval (estradiol 17-valerate)	Estradiol
climimycin	Clindamycin
Clinofug	Doxycycline
Clinovir	Medroxyprogesterone Acetate
Clonopin	Clonazepam
Clorazil	Clozapine
Clozaril	Clozapine
CN 3123	Trimethoprim
co-amoxiclav	Clavulanic Acid
Cobadex	Hydrocortisone
Cobutolin	Albuterol
Codicept	Codeine
Codinovo	Hydrocodone
Co-Ervonum	Ethinyl Estradiol
Co-Fram	Trimethoprim
Colifoam (hydrocortisone 21-acetate)	Hydrocortisone
Colimune	Cromolyn
Colisone	Prednisone
Colofoam (hydrocortisone 21-acetate)	Hydrocortisone
Colrex Compound	Codeine
Colrex Expectorant	Guaifenesin
Coly-Mycin S Otic	Neomycin
Coly-Mycin S Otic (hydrortisone acetate)	Hydrocortisone
Comox	Trimethoprim
Comox	Sulfamethoxazole
Compocillin-V	Penicillin V
compound 42	Warfarin
Compound 99638	Cefaclor
Compudose 365	Estradiol
Comycin	Tetracycline
Conceplan	Ethinyl Estradiol
Conceplan	Norethindrone
Conestron	Estrogens, Conjugated
Congestac	Guaifenesin
conjugated estrogenic hormones	Estrogens, Conjugated
Conludag	Norethindrone
Conova	Ethinodiol Diacetate
Conova	Ethinyl Estradiol
Consolan	Nabumetone
Constant-T	Theophylline
Contact	Acetaminophen
Contact	Guaifenesin
Contact	Phenylpropanolamine
Contratuss	Propoxyphene

NAME

Cortril Acetate-AS (hydrocortisone acetate)
 Cotrim
 Cotrim
 co-trimazine
 co-trimoxazole
 co-trimoxazole
 Cotrim-Puren
 Cotrim-Puren
 Coumadin
 CP 16171
 CP-16,171-85
 CP-16,533-1
 CP 45899
 CP-45899-2
 CP-51974-1
 CP 62993
 Cravit
 CRD-401
 Cremesone
 Crescormon
 Criseociclina
 Crispin
 cromoglycic acid
 cromolyn sodium
 cromolyn
 Cromovet
 Cronizat
 CS-514
 Curocef
 Curoxim
 Curretab
 Cutinolone Simple (triamcinolone acetonide)
 Cuxacillin
 Cuxanorm
N-cyano-*N'*-methyl-*N''*-[2-[[[5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine
 Cyantin
 Cyater
 Cyclacur (estradiol 17-valerate)
 Cycladol (compound with β -cyclodextrin)
 [R-[R*,R*-(E)]]-cyclic(L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-3-hydroxy-*N*,4-dimethyl-L-2-amino-6-octenoyl-L- α -aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl)
 cyclobenzaprine
 1,1-cyclobutanedicarboxylic acid platinum complex
 Cyclogesterin

MONOGRAPH

Hydrocortisone
 Sulfamethoxazole
 Trimethoprim
 Trimethoprim
 Sulfamethoxazole
 Trimethoprim
 Sulfamethoxazole
 Trimethoprim
 Warfarin
 Piroxicam
 Piroxicam
 Verapamil
 Sulbactam
 Sulbactam
 Sertraline
 Azithromycin
 Ofloxacin
 Diltiazem
 Hydrocortisone
 Somatropin
 Tetracycline
 Tramadol
 Cromolyn
 Cromolyn
 Cromolyn
 Cromolyn
 Nizatidine
 Pravastatin
 Cefuroxime
 Cefuroxime
 Medroxyprogesterone Acetate
 Triamcinolone
 Amoxicillin
 Atenolol
 Cimetidine

 Nitrofurantoin
 Terfenadine
 Estradiol
 Piroxicam
 Cyclosporine

 Cyclobenzaprine
 Carboplatin

 Estrogens, Conjugated

NAME	MONOGRAPH
<i>N</i> -[2-[4-[[[(cyclohexylamino) carbonyl]amino]sulfonyl]phenyl]ethyl]-5-methylpyrazinecarboxamide	Glipizide
1-cyclohexyl-3-[[<i>p</i> -[2-(5-methylpyrazine-carboxamido)ethyl]phenyl]sulfonyl]urea	Glipizide
cyclo-[[(<i>E</i>)-(2 <i>S</i> ,3 <i>R</i> ,4 <i>R</i>)-3-hydroxy-4-methyl-2-(methylamino)-6-octenyl]-L-2-aminobutyryl- <i>N</i> -methylglycyl- <i>N</i> -methyl-L-leucyl-L-valyl- <i>N</i> -methyl-L-leucyl-L-alanyl-D-alanyl- <i>N</i> -methyl-L-leucyl- <i>N</i> -methyl-L-leucyl- <i>N</i> -methyl-L-valyl]	Cyclosporine
Cyclomycin	Tetracycline
Cyclopar	Tetracycline
1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid	Ciprofloxacin
Cyclosa	Desogestrel
Cyclosa	Ethinyl Estradiol
cyclosporin A	Cyclosporine
Cycrin	Medroxyprogesterone Acetate
Cymbi	Ampicillin
Cystit	Nitrofurantoin
Cystospaz	Atropine
D 65MT	Alprazolam
D-365	Verapamil
Dacortin	Prednisone
Dafalgan	Acetaminophen
Dalacin C	Clindamycin
Dalacine	Clindamycin
Dalacin T	Clindamycin
Damide	Indapamide
Dansida	Ibuprofen
Dantafur	Nitrofurantoin
Danten	Phenytoin
Daonil	Glyburide
Darvocet N	Acetaminophen
Darvocet-N	Propoxyphene
Darvon	Propoxyphene
Darvon-N	Propoxyphene
Dasin	Aspirin
Datril	Acetaminophen
daturine	Atropine
Davoxin	Digoxin
Daypro	Oxaprozin
Decardil	Digoxin
Decortancyl	Prednisone
Decortin	Prednisone
Decortisyl	Prednisone
Decrelip	Gemfibrozil
de Graafina (estradiol 3-benzoate)	Estradiol
delta ¹ -dehydrocortisone	Prednisone
1-dehydro-6 α -methylhydrocortisone	Methylprednisolone
Dekortin	Prednisone

NAME

Delacillin
 Deladumone (estradiol 17-valerate)
 Delatuvel 2X (estradiol 17-valerate)
 Delcortin
 Delestrec (estradiol 17-undecanoate)
 Delestrogen (estradiol 17-valerate)
 Delgesic
 Delix
 Delphicort (triamcinolone acetonide)
 Delphimix
 delta E
 Delta-Corlin
 Delta-Cortelan (prednisone 21-acetate)
 deltacortisone
 Deltacortone
 Delta-Dome
 Delta Prenovis
 Deltasone
 Deltazen
 Deltison
 Deltra
 Demazin
 4'-demethylepipodophyllotoxin 9-[4,6-O-ethylidene- β -D-glucopyranoside]
 demethylepipodophyllotoxin ethylidene glucoside
 Democracin
 Demulen
 Demulen
 Denan
 Dentigoa
 Denyl Sodium
 9-deoxy-9a-methyl-9a-aza-9a-homoerythromycin A
 7-deoxy-7(S)-chlorolincomycin
 α -6-deoxy-5-hydroxytetracycline monohydrate
 α -6-deoxyoxytetracycline monohydrate
 Depakene
 Depakin
 Dépakine
 Depakote
 depGynogen (estradiol cypionate)
 Depo-Clinovir
 Depoestradiol (estradiol cypionate)
 Depofemin (estradiol cypionate)
 Depogen (estradiol cypionate)
 Depo-Medrate (methylprednisolone 21-acetate)
 Depo-Medrol (methylprednisolone 21-acetate)
 Depo-Medrone (methylprednisolone 21-acetate)
 Deponit
 Depo-Provera
 Deporone

MONOGRAPH

Amoxicillin
 Estradiol
 Estradiol
 Prednisone
 Estradiol
 Estradiol
 Aspirin
 Ramipril
 Triamcinolone
 Diclofenac
 Prednisone
 Prednisone
 Prednisone
 Prednisone
 Prednisone
 Prednisone
 Prednisone
 Prednisone
 Diltiazem
 Prednisone
 Prednisone
 Phenylpropanolamine
 Etoposide

 Etoposide

 Tetracycline
 Ethynodiol Diacetate
 Ethinyl Estradiol
 Simvastatin
 Ibuprofen
 Phenytoin
 Azithromycin

 Clindamycin
 Doxycycline
 Doxycycline
 Valproic Acid
 Valproic Acid
 Valproic Acid
 Valproic Acid
 Estradiol
 Medroxyprogesterone Acetate
 Estradiol
 Estradiol
 Estradiol
 Methylprednisolone
 Methylprednisolone
 Methylprednisolone
 Nitroglycerin
 Medroxyprogesterone Acetate
 Medroxyprogesterone Acetate

NAME	MONOGRAPH
DepoTestadiol (estradiol cypionate)	Estradiol
Deprancol	Propoxyphene
Deprex	Amitriptyline
Depromic	Propoxyphene
Derantel	Cephalexin
Derizene	Phenytoin
Dermabet	Betamethasone
Dermacort	Hydrocortisone
Dermairol (tretinoin)	Retinoic Acid
Dermocortal	Hydrocortisone
Dermolate	Hydrocortisone
Dermolen	Hydrocortisone
Dermosol (betamethasone 17-valerate)	Betamethasone
Dermovaleas (betamethasone 17-valerate)	Betamethasone
deschlorobionycin	Tetracycline
Descortancyl	Prednisone
Desdemin	Furosemide
desitriptilina	Nortriptyline
desmethylamitriptyline	Nortriptyline
Desogen	Desogestrel
Desogen	Ethinyl Estradiol
Develin	Propoxyphene
dextropropoxyphene	Propoxyphene
Dexacidin	Neomycin
Dexacidin	Polymyxin
Dexal	Ketoprofen
dexnorgestrel	Norgestrel
dextronorgestrel	Norgestrel
Dia-basan	Glyburide
Diabeta	Glyburide
diacepin	Diazepam
Diacycline	Tetracycline
Di-Adreson	Prednisone
Diafusor	Nitroglycerin
Dial-a-gesic	Acetaminophen
Dialar	Diazepam
2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine	Trimethoprim
<i>cis</i> -diammine(1,1-cyclobutanedicarboxylato)platinum(II)	Carboplatin
(SP-4-2)-diammine[1,1-cyclobutanedicarboxylato(2-)- <i>O,O'</i>]platinum	Carboplatin
Diazemuls	Diazepam
Dibactil	Trimethoprim
5 <i>H</i> -dibenz[<i>b,f</i>]azepine-5-carboxamide	Carbamazepine
3-(5 <i>H</i> -dibenz[<i>a,d</i>]cyclohepten-5-ylidene)- <i>N,N</i> -dimethyl-1-propanamine	Cyclobenzaprine
Diblocin	Doxazosin
1,3-di(2-carboxy-4-oxochromen-5-yloxy)-2-hydroxypropane	Cromolyn
1,3-di(2-carboxy-4-oxochromen-5-yloxy)propan-2-ol	Cromolyn

NAME	MONOGRAPH
[<i>o</i> -[(2,6-dichloroanilino)phenyl]acetic acid sodium salt	Diclofenac
9,21-dichloro-11 β ,17-dihydroxy-16 α -methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	Mometasone Furoate
(11 β ,16 α)-9,21-dichloro-17-[(2-furanylcarbonyl)oxy]-11-hydroxy-16-methylpregna-1,4-diene-3,20-dione	Mometasone Furoate
2-[(2,6-dichlorophenyl)amino]benzeneacetic acid monosodium salt	Diclofenac
(1 <i>S</i> - <i>cis</i>)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro- <i>N</i> -methyl-1-naphthalenamine	Sertraline
(1 <i>S</i> ,4 <i>S</i>)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro- <i>N</i> -methyl-1-naphthylamine	Sertraline
<i>cis</i> -1-[4-[[2-(2,4-dichlorophenyl)-2-(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)piperazine	Terconazole
Dichlorosal	Hydrochlorothiazide
Dichlotride	Hydrochlorothiazide
Dichronic	Diclofenac
Diclobenin	Diclofenac
diclofenac	Diclofenac
Diclo-Phlogont	Diclofenac
Diclo-Puren	Diclofenac
Diclord	Diclofenac
Dicloream	Diclofenac
Diclotride	Hydrochlorothiazide
Dicodid	Hydrocodone
Dicodrine	Hydrocodone
Dicromil	Desogestrel
Dicromil	Ethinyl Estradiol
Dicurin Procaine	Theophylline
dicyclomine	Dicyclomine
dicycloverin	Dicyclomine
(5 α ,6 α)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol	Codeine
[2 <i>R</i> -(2 <i>R</i> *,3 <i>S</i> *,4 <i>R</i> *,5 <i>R</i> *,8 <i>R</i> *,10 <i>R</i> *,11 <i>R</i> *,12 <i>S</i> *,13 <i>S</i> *,14 <i>R</i> *)]-13-[(2,6-dideoxy-3- <i>C</i> -methyl-3- <i>O</i> -methyl- α - <i>L</i> -ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- β - <i>D</i> -xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one	Azithromycin
(3 <i>R</i> *,4 <i>S</i> *,5 <i>S</i> *,6 <i>R</i> *,7 <i>R</i> *,9 <i>R</i> *,11 <i>R</i> *,12 <i>R</i> *,13 <i>S</i> *,14 <i>R</i> *)-4-[(2,6-dideoxy-3- <i>C</i> -methyl-3- <i>O</i> -methyl- α - <i>L</i> -ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(diethylamino)- β - <i>D</i> -xylo-hexopyranosyl]oxy]oxacyclotetra-decane-2,10-dione	Erythromycin

NAME	MONOGRAPH
(3 β ,5 β ,12 β)-3-[(<i>O</i> -2,6-dideoxy- β -D-ribohexopyranosyl-(14)- <i>O</i> -2,6-dideoxy- β -D-ribohexopyranosyl-(14))-2,6-dideoxy- β -D-ribohexopyranosyl]oxy]-12,14-dihydroxycard-20(22)-enolide	Digoxin
Diet Gard	Phenylpropanolamine
diethylaminocarbethoxybicyclohexyl	Dicyclomine
β -diethylaminoethyl 1-cyclohexylcyclohexanecarboxylate	Dicyclomine
β -diethylaminoethyl-1-cyclohexylhexahydrobenzoate	Dicyclomine
2-diethylaminoethyl diphenylacetate hydrochloride	Adiphenine
1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4- <i>b</i>]indole-1-acetic acid	Etodolac
Difacil hydrochloride	Adiphenine
Diffolisterol (estradiol 3-benzoate)	Estradiol
Diffumal	Theophylline
Difhydan	Phenytoin
Diflucan	Fluconazole
2,4-difluoro- α , α -bis(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)benzyl alcohol	Fluconazole
2-(2,4-difluorophenyl)-1,3-bis(1 <i>H</i> -1,2,4-triazol-1-yl)propan-2-ol	Fluconazole
α -(2,4-difluorophenyl)- α -(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)-1 <i>H</i> -1,2,4-triazole-1-ethanol	Fluconazole
Difolliculine (estradiol 3-benzoate)	Estradiol
Digacin	Digoxin
Dignover	Verapamil
Dihycon	Phenytoin
Di-Hydan	Phenytoin
Dihydan soluble	Phenytoin
3-(10,11-dihydro-5 <i>H</i> -dibenzo[<i>a,d</i>]cyclohepten-5-ylidene- <i>N,N</i> -dimethyl-1-propanamine	Amitriptyline
3-(10,11-dihydro-5 <i>H</i> -dibenzo[<i>a,d</i>]cyclohepten-5-ylidene- <i>N</i> -methyl-1-propanamine	Nortriptyline
3-(10,11-dihydro-5 <i>H</i> -dibenzo[<i>a,d</i>]cyclohepten-5-ylidene)- <i>N</i> -methylpropylamine	Nortriptyline
3,4-dihydrochlorothiazide	Hydrochlorothiazide
dihydrocodeinone	Hydrocodone
10,11-dihydro-5-(gamma-dimethylamino-propylidene)-5 <i>H</i> -dibenzo[<i>a,d</i>]cycloheptene	Amitriptyline
10,11-dihydro- <i>N,N</i> -dimethyl-5 <i>H</i> -dibenzo[<i>a,d</i>]cycloheptene-delta ^{5,γ} -propylamine	Amitriptyline
1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester	Nifedipine
3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-1 <i>H</i> -purine-2,6-dione	Pentoxifylline
3,7-dihydro-1,3-dimethyl-1 <i>H</i> -purine-2,6-dione	Theophylline
10,11-dihydro- <i>N</i> -methyl-5 <i>H</i> -dibenzo[<i>a,d</i>]cycloheptene-delta ^{5,γ} -propylamine	Nortriptyline

NAME	MONOGRAPH
3,4-dihydro-2-methyl-4-oxo- <i>N</i> -2-pyridyl-2 <i>H</i> -1,2-benzothiazine-3-carboxamide 1,1-dioxide	Piroxicam
dihydroequilenin	Estrogens, Conjugated
17 α -dihydroequilenin	Estrogens, Conjugated
dihydroequilin	Estrogens, Conjugated
17 α -dihydroequilin	Estrogens, Conjugated
dihydrofollicular hormone	Estradiol
dihydrofolliculin	Estradiol
dihydrohydroxycodone	Oxycodone
Dihydromenformon	Estradiol
10,11-dihydro-5-(3-methylaminopropylidene)-5 <i>H</i> -dibenzo[<i>a,d</i>][1,4]cycloheptene	Nortriptyline
Dihydrone	Oxycodone
dihydrotheelin	Estradiol
1,2-dihydroxy-3-(2-methoxyphenoxy)propane	Guaifenesin
dihydroxyestrin	Estradiol
17,21-dihydroxypregna-1,4-diene-3,11,20-trione	Prednisone
2,6-diisopropylphenol	Propofol
Dilabar	Captopril
Dilabid	Phenytoin
Diladel	Diltiazem
Di-Lan	Phenytoin
Dilanacin	Digoxin
Dilantin	Phenytoin
Di-Len	Phenytoin
Dilor G	Guaifenesin
Dimacol	Guaifenesin
Dilpral	Diltiazem
Dilrene	Diltiazem
diltiazem	Diltiazem
Dilzem	Diltiazem
Dilzene	Diltiazem
Dimapp	Promethazine
Dimenformon	Estradiol
Dimenformon benzoate (estradiol 3-benzoate)	Estradiol
Dimenformon Dipropionate (estradiol dipropionate)	Estradiol
diméprotane	Propoxyphene
Dimetapp	Phenylpropanolamine
5-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile	Verapamil
α -[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy- α -(1-methylethyl)benzeneacetonitrile	Verapamil
(+)-4-dimethylamino-1,2-diphenyl-3-methyl-2-propionyloxybutane	Propoxyphene
(+)- <i>cis</i> -5-[2-(dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(<i>p</i> -methoxyphenyl)-1,5-benzothiazepin-4(5 <i>H</i>)-one acetate (ester)	Diltiazem
3-[2-(dimethylamino)ethyl]- <i>N</i> -methyl-1 <i>H</i> -indole-5-methanesulfonamide	Sumatriptan

NAME	MONOGRAPH
1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol	Venlafaxine
α - <i>d</i> -4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate	Propoxyphene
<i>N</i> -(2'-dimethylamino-2'-methyl)ethylphenothiazine	Promethazine
10-(2-dimethylamino-2-methylethyl)phenothiazine	Promethazine
[<i>S</i> -(<i>R</i> *, <i>S</i> *)]- α -[2-(dimethylamino)-1-methylethyl]- α -phenylbenzeneethanol propanoate (ester)	Propoxyphene
<i>N</i> -[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]- <i>N'</i> -methyl-2-nitro-1,1-ethenediamine	Ranitidine
(\pm)-1-[α -[(dimethylamino)methyl]- <i>p</i> -methoxybenzyl]cyclohexanol	Venlafaxine
2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol	Tramadol
<i>N</i> -[2-[[[2-[(dimethylamino)methyl]-4-thiazolyl]methyl]thio]ethyl]- <i>N'</i> -methyl-2-nitro-1,1-ethenediamine	Nizatidine
4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide monohydrate	Doxycycline
5-(3-dimethylaminopropylidene)dibenzo[<i>a,d</i>][1,4]cycloheptadiene	Amitriptyline
5-(3-dimethylaminopropylidene)dibenzo[<i>a,e</i>]cycloheptatriene	Cyclobenzaprine
5-(γ -dimethylaminopropylidene)-5 <i>H</i> -dibenzo[<i>a,d</i>]-10,11-dihydrocycloheptene	Amitriptyline
1-(3-dimethylaminopropylidene)-2,3:6,7-dibenzo-4-suberene	Cyclobenzaprine
10-(2-dimethylaminopropyl)phenothiazine	Promethazine
[1 <i>S</i> -[1 α ,3 α ,7 β ,8 β (2 <i>S</i> *,4 <i>S</i> *),8a β]]-2,2-dimethylbutanoic acid 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2 <i>H</i> -pyran-2-yl)ethyl]-1-naphthalenyl ester	Simvastatin
2,2-dimethylbutyric acid 8-ester with (4 <i>R</i> ,6 <i>R</i>)-6-[2-[(1 <i>S</i> ,2 <i>S</i> ,6 <i>R</i> ,8 <i>S</i> ,8a <i>R</i>)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4-hydroxy-2 <i>H</i> -pyran-2-one	Simvastatin
<i>N,N</i> -dimethyl-5 <i>H</i> -dibenzo[<i>a,d</i>]cyclohepten-delta ^{5,γ} -propylamine	Cyclobenzaprine
α ¹ -[[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol	Albuterol
(<i>S</i>)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5,-thiadiazol-3-yl]oxy]-2-propanol	Timolol
(5 α ,17 β)- <i>N</i> -(1,1-dimethylethyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide	Finasteride

NAME	MONOGRAPH
α -[4-(1,1-dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl)-1-piperidinebutanol	Terfenadine
6-[O-(1,1-dimethylethyl)-D-serine]-10-deglycinamideluteinizing hormone-releasing factor (pig) 2-(aminocarbonyl)hydrazide	Goserelin
(E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalene methanamine	Terbinafine
2 β ,6 α -dimethyl-8 α -(2-methyl-1-oxobutoxy)-mevinic acid lactone	Lovastatin
3,7-dimethyl-1-(5-oxohexyl)-1 <i>H</i> ,3 <i>H</i> -purin-2,6-dione	Pentoxifylline
3,3-dimethyl-7-oxo-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	Penicillin V
(2 <i>S</i> -cis)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide	Sulbactam
5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid	Gemfibrozil
3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid	Retinoic Acid
3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-cis-4-trans-6-trans-8-trans-nonatetraenoic acid (isotretinoin)	Retinoic Acid
1,3-dimethylxanthine	Theophylline
2,2-dimethyl-5-(2,5-xylyloxy)valeric acid	Gemfibrozil
Dinarkon	Oxycodone
Diocimex	Doxycycline
Diocodal	Naproxen
Diocyclin	Tetracycline
Diocyl Hydrochloride	Dicyclomine
Dioderm	Hydrocortisone
Diogyn	Estradiol
Diogyn B (estradiol 3-benzoate)	Estradiol
Diogyn E	Ethinyl Estradiol
Diogynets	Estradiol
Diosmal	Nabumetone
Diovocilin (estradiol dipropionate)	Estradiol
Dioxanin	Digoxin
Dipam	Diazepam
Diphacil hydrochloride	Adiphenine
Diphantoine	Phenytoin
Diphenin	Phenytoin
Diphenine Sodium	Phenytoin
Diphentoin	Phenytoin
diphenylacetyldiethylaminoethanol hydrochloride	Adiphenine
Diphenylan Sodium	Phenytoin
diphenyl-(2-chlorophenyl)-1-imidazolylmethane	Clotrimazole
diphenylhydantoin	Phenytoin
5,5-diphenyl-2,4-imidazolidinedione	Phenytoin
4,5-diphenyl-2-oxazolepropanoic acid	Oxaprozin

NAME	MONOGRAPH
4,5-diphenyl-2-oxazolepropionic acid	Oxaprozin
β -(4,5-diphenyloxazol-2-yl)propionic acid	Oxaprozin
(+)-1,2-diphenyl-2-propionyloxy-3-methyl-4-dimethylaminobutane	Propoxyphene
Diphergan	Promethazine
Diprivan	Propofol
Diproderm (betamethasone 17,21-dipropionate)	Betamethasone
Diprolene (betamethasone dipropionate)	Betamethasone
Diprophos (betamethasone 17,21-dipropionate)	Betamethasone
Diprosis (betamethasone 17,21-dipropionate)	Betamethasone
Diprosone (betamethasone 17,21-dipropionate)	Betamethasone
Direma	Hydrochlorothiazide
Dirox	Acetaminophen
Disalunil	Hydrochlorothiazide
Discoïd	Furosemide
Discotrine	Nitroglycerin
disodium cromoglycate	Cromolyn
Disoprivan	Propofol
disopropol	Propofol
Disprol	Acetaminophen
Dispromil	Famotidine
Distaclor	Cefaclor
Distakaps V-K	Penicillin V
Distaquaine V	Penicillin V
Distaquaine V-K	Penicillin V
Distaxid	Nizatidine
Di-Syntramine	Dicyclomine
Ditate (estradiol 17-valerate)	Estradiol
Diu-melusin	Hydrochlorothiazide
Diural	Furosemide
divalproex sodium	Valproic Acid
Divercillin	Ampicillin
Dixina	Digoxin
DL-8280	Ofloxacin
DMSC	Doxycycline
Dokim	Digoxin
Doktacillin	Ampicillin
Dolac	Ketorolac
Dolene	Propoxyphene
Dolgin	Ibuprofen
Dolgirid	Ibuprofen
Dolgit	Ibuprofen
Doliprane	Acetaminophen
Dolo-Dolgit	Ibuprofen
Dolobasan	Diclofenac
Dolocap	Propoxyphene
Dolocyl	Ibuprofen
Dolonil	Atropine
Doloxene	Propoxyphene
Dolprone	Acetaminophen
Domarax	Carisoprodol
Dome-Cort	Hydrocortisone

NAME	MONOGRAPH
Duracroman	Cromolyn
Duradoxal	Doxycycline
Durafurid	Furosemide
Duraglucon	Glyburide
Duramax	Aspirin
Duranifin	Nifedipine
Durapaediat (erythromycin ethylsuccinate)	Erythromycin
Durapental	Pentoxifylline
Duraphyl	Theophylline
Duraphyllin	Theophylline
Durapro	Oxaprozin
Duraprost	Oxaprozin
Duratriamet	Sulfamethoxazole
Duratriamet	Trimethoprim
Duravolten	Diclofenac
Duricef	Cefadroxil
Duteplase	Alteplase
Duxima	Cefuroxime
Dyazide	Hydrochlorothiazide
Dyazide	Triamterene
Dyloform	Ethinyl Estradiol
Dymadon	Acetaminophen
Dynamos	Digoxin
Dyren	Triamterene
Dyrenium	Triamterene
Dysmenalgit	Naproxen
Dyspamet	Cimetidine
Dyspas	Dicyclomine
Dytac	Triamterene
E-265	Tramadol
Easprin	Aspirin
E-base	Erythromycin
Ebufac	Ibuprofen
Ecazide	Captopril
Eclabron	Guaifenesin
ECM	Aspirin
Ecodipin	Nifedipine
Ecodox	Doxycycline
Ecofenac	Diclofenac
Ecotrin	Aspirin
Ecoval 70 (betamethasone 17-valerate)	Betamethasone
Ecovent	Albuterol
ECP (estradiol cypionate)	Estradiol
ED	Ethinodiol Diacetate
Edalene	Cimetidine
Edolan	Etodolac
E.E.S. (erythromycin ethylsuccinate)	Erythromycin
Efalexin	Cephalexin
Efcorbin	Hydrocortisone
Efcorlin (hydrocortisone 21-acetate)	Hydrocortisone
Efcortelan	Hydrocortisone
EF-Cortelan	Hydrocortisone

NAME	MONOGRAPH
EF-Cortelan Soluble (hydrocortisone 21-sodium succinate)	Hydrocortisone
Efcortelin	Hydrocortisone
Efcortisol (hydrocortisone 21-phosphate disodium salt)	Hydrocortisone
Eferox	Levothyroxine
Effederm (tretinoin)	Retinoic Acid
Effekton	Diclofenac
Effexor	Venlafaxine
Efpenix	Amoxicillin
Eismycin	Mupirocin
Ekko	Phenytoin
Ekomine	Atropine
Elavil	Amitriptyline
Elazor	Fluconazole
Eldecort	Hydrocortisone
Elisor	Pravastatin
Elixicon	Theophylline
Elixophyllin	Theophylline
Elobact (cefuroxime axetil)	Cefuroxime
Elocon	Mometasone Furoate
Eltrianyl	Sulfamethoxazole
Eltrianyl	Trimethoprim
Eltroxin	Levothyroxine
Emgel	Erythromycin
Emodin	Ibuprofen
Emotival	Lorazepam
Empecid	Clotrimazole
Empirin	Aspirin
Empirin	Codeine
Empracet	Acetaminophen
EMU	Erythromycin
E-Mycin	Erythromycin
E-Mycin E (erythromycin ethylsuccinate)	Erythromycin
Enacard	Enalapril
enalapril	Enalapril
Enaloc	Enalapril
Enantone	Leuprolide
Enap	Enalapril
Enapren	Enalapril
Encaprin	Aspirin
Encorton	Prednisone
Endecon	Acetaminophen
Endecon	Phenylpropanolamine
Endep	Amitriptyline
Etrafon	Amitriptyline
Endomixin	Neomycin
Endural	Furosemide
Endydol	Aspirin
Enelfa	Acetaminophen
Eneril	Acetaminophen
Englate	Theophylline

NAME

Enterfram
 Enterosarine
 ENTEX
 ENTEX
 Entrophen
 Entyderma
 Ep
 Epanutin
 Epatec
 EPEG
 Epi-Aberel (tretinoin)
 Epicort
 Epifoam (hydrocortisone acetate)
 3,17-epidihydroxyestratriene
 Epilim
 Epitol
 Epo
 Epoade
 Epobron
 epoetin
 Epogen
 5 β ,20-epoxy-1,2 α ,4,7 β ,10 β ,13 α -hexahydroxy-
 tax-11-en-9-one 4,10-diacetate 2-benzoate
 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-
 phenylisoserine
 4,5-epoxy-14-hydroxy-3-methoxy-17-
 methylmorphinan-6-one
 4,5-epoxy-3-methoxy-17-methylmorphinan-6-one
 6 β ,7 β -epoxy-1 α *H*,5 α *H*-tropan-3 α -ol (—)-tropate
 (ester)
 6 β ,7 β -epoxy-3 α -tropanyl *S*-(—)-tropate
 6,7-epoxytropine tropate
 Eprex
 eptastatin
 Eptoin
 Equagesic
 Equicol
 equilenin
 equilin
 Equiproxen
 ER 115
 Erantin
 Erasid (erythromycin acistrate)
 Eratrex (erythromycin stearate)
 Erazon
 Ergenyl
 Eridan
 Eriscel
 Eritrocina
 Eritroger (erythromycin estolate)
 Ermysin
 Eromycin (erythromycin estolate)

MONOGRAPH

Neomycin
 Aspirin
 Guaifenesin
 Phenylpropanolamine
 Aspirin
 Beclomethasone Dipropionate
 Epoetin
 Phenytoin
 Ketoprofen
 Etoposide
 Retinoic Acid
 Hydrocortisone
 Hydrocortisone
 Estradiol
 Valproic Acid
 Carbamazepine
 Epoetin
 Epoetin
 Ibuprofen
 Epoetin
 Epoetin
 Epoetin
 Paclitaxel

 Oxycodone

 Hydrocodone
 Scopolamine

 Scopolamine
 Scopolamine
 Epoetin
 Pravastatin
 Phenytoin
 Aspirin
 Guaifenesin
 Estrogens, Conjugated
 Estrogens, Conjugated
 Naproxen
 Temazepam
 Propoxyphene
 Erythromycin
 Erythromycin
 Piroxicam
 Valproic Acid
 Diazepam
 Erythromycin
 Erythromycin
 Erythromycin
 Erythromycin
 Erythromycin

NAME	MONOGRAPH
Errolon	Furosemide
Ery Derm	Erythromycin
Ery-Tab	Erythromycin
ERYC	Erythromycin
Erycen	Erythromycin
Erycette	Erythromycin
Erycin	Erythromycin
Erycinum	Erythromycin
EryDerm	Erythromycin
Erygel	Erythromycin
Eryliquid (erythromycin ethylsuccinate)	Erythromycin
Erymax	Erythromycin
Erypar (erythromycin stearate)	Erythromycin
EryPed (erythromycin ethylsuccinate)	Erythromycin
Erypo	Epoetin
Eryprim (erythromycin stearate)	Erythromycin
Erythro ES (erythromycin ethylsuccinate)	Erythromycin
Erythro S (erythromycin stearate)	Erythromycin
Erythro S	Erythromycin
Erythro	Erythromycin
Erythro-Holz (erythromycin ethylsuccinate)	Erythromycin
Erythrocin (erythromycin stearate)	Erythromycin
Erythrocin Lactobionate (erythromycin lactobionate)	Erythromycin
Erythrogan	Erythromycin
Erythroguent	Erythromycin
Erythromast 36	Erythromycin
Erythromid	Erythromycin
Erythromycin A	Erythromycin
Erythroped (erythromycin ethylsuccinate)	Erythromycin
erythropoietin	Epoetin
1-165-erythropoietin (human clone lambdaHEPOFL 13 protein moiety), glycoform α	Epoetin
1-165-erythropoietin (human clone lambdaHEPOFL 13 protein moiety), glycoform β	Epoetin
1-165-erythropoietin (human clone lambdaHEPOFL 13 protein moiety), glycoform β	Epoetin
1-165-erythropoietin (human clone lambdaHEPOFL 13 protein moiety), glycoform α	Epoetin
Erytrarco	Erythromycin
ESF	Epoetin
Esidrex	Hydrochlorothiazide
Esidrix	Hydrochlorothiazide
Esimil	Hydrochlorothiazide
Esinol (erythromycin ethylsuccinate)	Erythromycin
Eskacillin V	Penicillin V
Espo	Epoetin
Estigyn	Ethinyl Estradiol

NAME	MONOGRAPH
Estinyl	Ethinyl Estradiol
Estomicina	Erythromycin
Eston-B (estradiol 3-benzoate)	Estradiol
Eston-E	Ethinyl Estradiol
Estopherol	Ethinyl Estradiol
Estrace	Estradiol
Estradep (estradiol cypionate)	Estradiol
Estraderm	Estradiol
estradiol	Estrogens, Conjugated
α -estradiol	Estradiol
β -estradiol	Estradiol
17 α -estradiol	Estrogens, Conjugated
1,3,5:10,6,8-estrapentaen-3-ol-17-one (equilenin)	Estrogens, Conjugated
estra-1,3,5-(10),7-tetraene-3,17-diol (17 α - dihydroequilin)	Estrogens, Conjugated
1,3,5,7-estratetraen-3-ol-17-one (equilin)	Estrogens, Conjugated
(17 β)-estra-1,3,5(10)-triene-3,17-diol	Estradiol
(17 β)-estra-1,3,5(10)-triene-3,17-diol (estradiol)	Estrogens, Conjugated
1,3,5-estratrien-3-ol-17-one (estrone)	Estrogens, Conjugated
Estrifol	Estrogens, Conjugated
Estroclim	Estradiol
Estrofem (estradiol cypionate)	Estradiol
estrone	Estrogens, Conjugated
Estrovite	Estradiol
Estted	Ethinyl Estradiol
Etalontin (norethindrone acetate)	Norethindrone
Etheophyl	Theophylline
Ethidol	Ethinyl Estradiol
17-ethinylestradiol	Ethinyl Estradiol
Ethinyl-Oestradiol	Ethinyl Estradiol
ethinyloestradiol	Ethinyl Estradiol
(S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L- alanyl]-L-proline	Enalapril
[2S-[1[R*(R*)],2 α ,3 α ,6 α]]-1-[2-[[1- (ethoxycarbonyl)-3-phenylpropyl]amino]-1- oxopropyl]octahydrocyclopenta[b]pyrrole-2- carboxylic acid	Ramipril
[3S-[2[R*(R*)],3R*]]-2-[2-[[1-ethoxycarbonyl- 3-phenylpropyl]amino]-1-oxopropyl]- 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid	Quinapril
[S-(R*,R*)]-3-[[1-(ethoxycarbonyl)-3- phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo- 1H-1-benzazepine-1-acetic acid	Benazepril
11-[N-(ethoxycarbonyl)-4-piperidylidene]-8- chloro-6,11-dihydro-5H- benzo[5,6]cyclohepta[1,2-b]pyridine	Loratadine
Ethril (erythromycin stearate)	Erythromycin
Ethryn (erythromycin stearate)	Erythromycin
Ethy 11	Ethinyl Estradiol

NAME	MONOGRAPH
13 β -ethyl-17 α -ethynyl-17 β -hydroxygon-4-ene-3-one	Norgestrel
13-ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one	Norgestrel
9[(4,6- <i>O</i> -ethylidene- β -D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3- <i>d</i>]-1,3-dioxol-6(5a <i>H</i>)-one	Etoposide
(17 α)-13-ethyl-11-methylene-18,19-dinorpregn-4-en-20-yn-17-ol	Desogestrel
ethynodiol	Ethynodiol Diacetate
ethynylestradiol	Ethynyl Estradiol
17 α -ethynyl-1,3,5(10)-estratriene-3,17 β -diol	Ethynyl Estradiol
17 α -ethynyl-4-estrene-3 β ,17 β -diol diacetate	Ethynodiol Diacetate
17 α -ethynyl-18-homo-19-nortestosterone	Norgestrel
17 α -ethynyl-18-methyl-11-methylene- δ^4 -estren-17 β -ol	Desogestrel
17 α -ethynyl-19-norandrost-4-ene-3 β ,17 β -diol diacetate	Ethynodiol Diacetate
17 α -ethynyl-19-nortestosterone	Norethindrone
Eticyclin	Ethynyl Estradiol
Eticylol	Ethynyl Estradiol
Etinestrol	Ethynyl Estradiol
Etinestryl	Ethynyl Estradiol
Etinoestryl	Ethynyl Estradiol
Etivex	Ethynyl Estradiol
etodolic acid	Etodolac
Eubine	Oxycodone
Euclamin	Glyburide
Eucodal	Oxycodone
Eudigox	Digoxin
Eudyna (tretinoin)	Retinoic Acid
Euflex	Flutamide
Euglucon	Glyburide
Euhypnos	Temazepam
Euipnos	Temazepam
Eukodal	Oxycodone
Eulexin	Flutamide
Eu-Med	Acetaminophen
Eumydrin	Atropine
Euphyllin	Theophylline
Euphylline L.A.	Theophylline
Euphyllong	Theophylline
Euplit	Amitriptyline
Eupragin (erythromycin estolate)	Erythromycin
Eureceptor	Cimetidine
Eurekene	Valproic Acid
Eurosan	Diazepam
Eusaprim	Sulfamethoxazole
Eusaprim	Trimethoprim
Eutagen	Oxycodone
Eutensin	Furosemide

NAME

Euthyrox
 Eutocol (estradiol hemisuccinate)
 Euvaderm (betamethasone 17-benzoate)
 Evacalm
 Evacort
 Evinopon
 Evorel
 Exdol
 Exocin
 Extracort (triamcinolone acetonide)
 Eye-Cort
 Falcopen-V
 Famodil
 Famodine
 Famosan
 Famoxal
 Fanosin
 Farexin
 Fergon 500
 Fargan
 Farlital
 Fastum
 Faustan
 FB 5097
 Febrilex
 Fectrim
 Fectrim
 Fedahist
 Feldene
 Fellozine
 Femadol
 Femadon
 Femestral
 Femestrone (estradiol 3-benzoate)
 Feminone
 Femodene
 Femovan
 Femulen
 Fenacilin
 Fenazil
 Fenbid
 Fenergan
 Fenospen
 Fenoxypen
 Fernisone
 Fibonel
 Fibrinokinase
 Ficortril
 Finastid
 Finimal
 Finlepsin
 Fivent

MONOGRAPH

Levothyroxine
 Estradiol
 Betamethasone
 Diazepam
 Hydrocortisone
 Diclofenac
 Estradiol
 Acetaminophen
 Ofloxacin
 Triamcinolone
 Hydrocortisone
 Penicillin V
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Cephalexin
 Cephalexin
 Promethazine
 Medroxyprogesterone Acetate
 Ketoprofen
 Diazepam
 Clotrimazole
 Acetaminophen
 Sulfamethoxazole
 Trimethoprim
 Guaifenesin
 Piroxicam
 Promethazine
 Propoxyphene
 Ibuprofen
 Estradiol
 Estradiol
 Ethinyl Estradiol
 Ethinyl Estradiol
 Ethinyl Estradiol
 Ethynodiol Diacetate
 Penicillin V
 Promethazine
 Ibuprofen
 Promethazine
 Penicillin V
 Penicillin V
 Prednisone
 Famotidine
 Alteplase
 Hydrocortisone
 Finasteride
 Acetaminophen
 Carbamazepine
 Cromolyn

NAME

FK-027
 Flanax
 Flexal
 Flexartal
 Flexeril
 Flexiban
 Flobacin
 Flociprin
 Floginax
 Flogobene
 Flormidal
 Floxil
 Floxin
 flubenisolone
 Flucinom
 Fluctin
 Fludex
 Flugeril
 Fugerel
 fluindostatin
 1-(*p*-fluorobenzyl)-2-[[1-(*p*-methoxyphenethyl)-4-piperidyl] amino]benzimidazole
 (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid
 delta¹-9α-fluoro-16α-hydroxyhydrocortisone
 9-αfluoro-16α-hydroxyprednisolone
 9-αfluoro-16β-methylprednisolone
 [*R**,*S**-(*E*)]-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-2,5-dihydroxy-6-heptenoic acid, monosodium salt
 1-[(4-fluorophenyl)methyl]-*N*-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidiny]-1*H*-benzimidazol-2-amine
 9-fluoro-11,16,17,21-tetrahydroxypregna-1,4-diene-3,20-dione
 9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione
 Fluoxeren
 Flurobate (betamethasone 17-benzoate)
 Flutex (triamcinolone acetonide)
 fluvastatin
 Fluvin
 Follicormon (estradiol 3-benzoate)
 Follidrin (estradiol 3-benzoate)
 Fontex
N-formimidoylthienamycin monohydrate
 Fortam
 Fortaz
 Fortum
 Foxetin
 FPL 670

MONOGRAPH

Cefixime
 Naproxen
 Carisoprodol
 Carisoprodol
 Cyclobenzaprine
 Cyclobenzaprine
 Ofloxacin
 Ciprofloxacin
 Naproxen
 Piroxicam
 Midazolam
 Ofloxacin
 Ofloxacin
 Betamethasone
 Flutamide
 Fluoxetine
 Indapamide
 Flutamide
 Flutamide
 Flutamide
 Fluvastatin
 Astemizole

 Ofloxacin

 Triamcinolone
 Triamcinolone
 Betamethasone
 Fluvastatin

 Astemizole

 Triamcinolone

 Betamethasone

 Fluoxetine
 Betamethasone
 Triamcinolone
 Fluvastatin
 Hydrochlorothiazide
 Estradiol
 Estradiol
 Fluoxetine
 Imipenem
 Ceftazidime
 Ceftazidime
 Ceftazidime
 Fluoxetine
 Cromolyn

NAME

Glycolande
 glydiazinamide
 Glytheonate
 GP 25840
 GR-20263
 GR 33343 G
 GR 33343 X
 GR 38032F
 GR 43175
 GR 43175C
 GR-122311X
 GR-C507/75
 Graafina (estradiol 3-benzoate)
 Grampenil
 Granudoxy
 Grinsil
 Gorm
 growth hormone
 GS-3065
 GSH
 GTN
 guaiacol glyceryl ether
 guaiacuran
 guaiacyl glyceryl ether
 Guaiamar
 guaiphenesin
 guaithylline
 Guayanesin
 Guiatuss
 Guicitrina
 Gyne-Lotrimin
 Gynécormone (estradiol 3-benzoate)
 Gynera
 Gynergon
 Gynestrel
 Gynoestryl
 Gynofug
 Gynogen LA (estradiol 17-valerate)
 Gynolett
 Gyno-Terazol
 Gynovlar (norethindrone acetate)
 H 93/26 succinate
 H 168/68
 Halodrin
 Haltran
 Harmar
 Harvatrate
 HB 419
 Hc45 (hydrocortisone 21-acetate)
 Headache Strength Allerest
 Hedex
 Heitrin

MONOGRAPH

Glyburide
 Glipizide
 Theophylline
 Diclofenac
 Ceftazidime
 Salmeterol
 Salmeterol
 Ondansetron
 Sumatriptan
 Sumatriptan
 Ranitidine
 Ondansetron
 Estradiol
 Ampicillin
 Doxycycline
 Amoxicillin
 Somatropin
 Somatropin
 Doxycycline
 Somatropin
 Nitroglycerin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Ampicillin
 Clotrimazole
 Estradiol
 Ethinyl Estradiol
 Estradiol
 Naproxen
 Estradiol
 Ibuprofen
 Estradiol
 Ethinyl Estradiol
 Terconazole
 Norethindrone
 Metoprolol
 Omeprazole
 Ethinyl Estradiol
 Ibuprofen
 Propoxyphene
 Atropine
 Glyburide
 Hydrocortisone
 Acetaminophen
 Acetaminophen
 Terazosin

NAME

Helicon
 Helvamox
 Helvecyclin
 Helveprim
 Hemi-Daonil
 hemopietine
 Herbesser
 hexadeca- μ -hydroxytetracosahydroxy[μ_8 -[1,3,4,6-
 tetra-*O*-sulfo- β -D-fructofuranosyl- α -D-
 glucopyranoside tetrakis(hydrogen sulfato)
 (8-)]hexadecaaluminum
 Hexadilat
 Hexagastron
 1,2,6,7,8,8a-hexahydro- β , δ -dihydroxy-
 2,6-dimethyl-8-(2-methyl-1-oxobutoxy)-
 1-naphthaleneheptanoic acid
 δ -lactone
 (1*S*,3*R*,7*S*,8*S*,8*aR*)-1,2,3,7,8,8a-hexahydro-3,7-
 dimethyl-8-[2-[(2*R*,4*R*)-tetrahydro-4-hydroxy-
 6-oxo-2*H*-pyran-2-yl]ethyl]-1-naphthalenyl (*S*)-
 2-methylbutyrate
 11,12,13,14,15,16-hexahydro-3-hydroxy-13-
 methyl-17*H*-cyclopenta[a]phenanthren-17-one
 (equilenin)
 [1*S*-[1 α -(β *S*^{*}, δ *S*^{*}),2 α ,6 α ,8 β (*R*^{*}),8 α]]-
 1,2,6,7,8,8a-hexahydro- β , δ ,6-trihydroxy-2-
 methyl-8-(2-methyl-1-oxobutoxy)-1-
 naphthaleneheptanoic acid monosodium salt
 Hexatrione (triamcinolone hexacetonide)
 HF-1854
 HGH
 H.H. 25/25
 H.H. 50/50
 Hiberna
 Hiconcil
 Hidantal
 Hidro-Colisina
 Hydroestron (estradiol 3-benzoate)
 Hidrononol
 Hipoartel
 Hismanal
 Histabid Duracap
 Histalet Forte
 Histalet X Tablets
 Histamen
 Histaminos
 Histazol
 HOE 280
 Hoe 498
 Homoolan
 Horizon

MONOGRAPH

Aspirin
 Amoxicillin
 Tetracycline
 Trimethoprim
 Sulfamethoxazole
 Glyburide
 Epoetin
 Diltiazem
 Sucralfate

 Nifedipine
 Sucralfate
 Lovastatin

 Lovastatin

 Estrogens, Conjugated

 Pravastatin

 Triamcinolone
 Clozapine
 Somatropin
 Hydrochlorothiazide
 Hydrochlorothiazide
 Promethazine
 Amoxicillin
 Phenytoin
 Hydrocortisone
 Estradiol
 Hydrochlorothiazide
 Enalapril
 Astemizole
 Phenylpropanolamine
 Phenylpropanolamine
 Guaifenesin
 Astemizole
 Astemizole
 Astemizole
 Ofloxacin
 Ramipril
 Acetaminophen
 Diazepam

NAME	MONOGRAPH
Hormezon (betamethasone 17-valerate)	Betamethasone
Hormogynon (estradiol 3-benzoate)	Estradiol
Hostacortin (prednisone 21-acetate)	Prednisone
Hostacyclin	Tetracycline
HR-756	Cefotaxime
human growth hormone	Somatropin
Humatrope	Somatropin
Huminsulin	Insulin
Humulin	Insulin
Humulina	Insulin
HVB	Hydrocortisone
Hycodan	Hydrocodone
Hycomine	Acetaminophen
Hycomine	Hydrocodone
Hycomine	Phenylpropanolamine
Hycorace (hydrocortisone 21-sodium succinate)	Hydrocortisone
Hycotuss	Guaifenesin
Hycotuss	Hydrocodone
Hydantin	Phenytoin
Hydantol	Phenytoin
Hydracort	Hydrocortisone
Hydramycin	Doxycycline
Hydril	Hydrochlorothiazide
Hydrin-2 (hydrocortisone acetate)	Hydrocortisone
Hydro-Adreson	Hydrocortisone
Hydro-Aquil	Hydrochlorothiazide
Hydrocal (hydrocortisone 21-acetate)	Hydrocortisone
hydrocodone	Hydrocodone
Hydrocort	Hydrocortisone
Hydrocortisat (hydrocortisone acetate)	Hydrocortisone
hydrocortisone TBA (hydrocortisone tebutate)	Hydrocortisone
Hydrocortistab (hydrocortisone 21-acetate)	Hydrocortisone
Hydrocortisyl	Hydrocortisone
Hydrocortone	Hydrocortisone
Hydrocortone Acetate (hydrocortisone 21-acetate)	Hydrocortisone
Hydrocortone Phosphate (hydrocortisone 21-phosphate disodium salt)	Hydrocortisone
Hydrocortone Sodium Phosphate	Hydrocortisone
Hydrocortone TBA (hydrocortisone tebutate)	Hydrocortisone
Hydro-Diuril	Hydrochlorothiazide
Hydrokon	Hydrocodone
Hydroled	Furosemide
Hydropres	Hydrochlorothiazide
Hydro-rapid	Furosemide
Hydrosaluric	Hydrochlorothiazide
Hydrothide	Hydrochlorothiazide
<i>p</i> -hydroxyacetanilide	Acetaminophen
4'-hydroxyacetanilide	Acetaminophen
6-(<i>p</i> -hydroxy- α -aminophenylacetamido)penicillanic acid	Amoxicillin
α -hydroxy- β -aminopropylbenzene hydrochloride	Phenylpropanolamine
<i>p</i> -hydroxyampicillin	Amoxicillin

NAME	MONOGRAPH
<i>p</i> -hydroxycephalexine monohydrate	Cefadroxil
3 β -hydroxycompactin sodium salt	Pravastatin
17-hydroxycorticosterone	Hydrocortisone
1-(4'-hydroxy-3'-coumarinyl)-1-phenyl-3-butanone	Warfarin
5-hydroxy- α -6-deoxytetracycline monohydrate	Doxycycline
3-hydroxydiazepam	Temazepam
14-hydroxydihydrocodeinone	Oxycodone
<i>O</i> -(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine monosodium salt	Levothyroxine
3-hydroxyestra-1,3,5,7,9-pentaen-17-one (equilenin)	Estrogens, Conjugated
3-hydroxyestra-1,2,5(10),7-tetraen-17-one (equilin)	Estrogens, Conjugated
3-hydroxyestra-1,3,5(10)-trien-17-one (estrone)	Estrogens, Conjugated
9-[(2-hydroxyethoxy)methyl]guanine	Acyclovir
3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	Clavulanic Acid
[5 <i>R</i>]-[5 α ,6 α (<i>R</i> *)]-6-(1-hydroxyethyl)-3-[[2-[(iminomethyl)amino]ethyl]thio]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate	Imipenem
delta ¹ -16 α -hydroxy-9 α -fluorohydrocortisone	Triamcinolone
16 α -hydroxy-9 α -fluoroprednisolone	Triamcinolone
4-hydroxy-3-hydroxymethyl- α -[(<i>tert</i> -butylamino)methyl]benzyl alcohol	Albuterol
2-[<i>p</i> -[2-hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide	Atenolol
(8 <i>R</i>)-3 α -hydroxy-8-isopropyl-1 α <i>H</i> ,5 α <i>H</i> -tropanium bromide (\pm)-tropate	Ipratropium Bromide
α -(hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester	Atropine
<i>endo</i> -(\pm)- α -(hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester	Atropine
[7(<i>S</i>)-(1 α ,2 β ,4 β ,5 α ,7 β)]- α -(hydroxymethyl)benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo[3,3,1,0 ^{2,4}]non-7-yl ester	Scopolamine
4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide	Atenolol
17-hydroxy-6 α -methylpregn-4-ene-3,20-dione acetate	Medroxyprogesterone Acetate
4-hydroxy-2-methyl- <i>N</i> -2-pyridinyl-2 <i>H</i> -1,2-benzothiazine-3-carboxamide 1,1-dioxide	Piroxicam
(17 α)-17-hydroxy-19-norpregn-4-en-20-yn-3-one	Norethindrone
4-hydroxy-3-(3-oxo-1-phenylbutyl)-2 <i>H</i> -1-benzopyran-2-one	Warfarin
(<i>endo</i> , <i>syn</i>)-(\pm)-3-(3-hydroxy-1-oxo-2-phenylpropoxy)-8-methyl-8-(1-methylethyl)-6-azoniabicyclo[3.2.1]octane bromide	Ipratropium Bromide
<i>N</i> -(4-hydroxyphenyl)acetamide	Acetaminophen

NAME	MONOGRAPH
(—)-6-[2-amino-2-(<i>p</i> -hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	Amoxicillin
(±)-4-hydroxy- α '-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]-1,3-benzenedimethanol	Salmeterol
(±)-4-hydroxy- α '-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]- <i>m</i> -xylene- α , α '-diol	Salmeterol
5,5'-[(2-hydroxy-1,3-propanediyl)bis(oxy)]bis[4-oxo-4 <i>H</i> -1-benzopyran-2-carboxylic acid]	Cromolyn
5,5'-(2-hydroxytrimethylenedioxy)bis(4-oxochromene-2-carboxylic acid)	Cromolyn
5,5'-[(2-hydroxytrimethylene)dioxy]bis(4-oxo-4 <i>H</i> -1-benzopyran-2-carboxylic acid)	Cromolyn
Hydrozide	Hydrochlorothiazide
hyoscine	Scopolamine
hyoscyamine	Atropine
Hypertil	Captopril
Hy-Phen	Acetaminophen
Hy-Phen	Hydrocodone
Hypnovel	Midazolam
hypophyseal growth hormone	Somatropin
Hypothiazide	Hydrochlorothiazide
Hypurin	Insulin
Hysron	Medroxyprogesterone Acetate
Hytone	Hydrocortisone
Hytracin	Terazosin
Hytrin	Terazosin
Hytrinex	Terazosin
Ibiamox	Amoxicillin
Ibilex	Cephalexin
Ibinolo	Atenolol
Ibu-Attritin	Ibuprofen
Ibumetin	Ibuprofen
Ibuprocin	Ibuprofen
Ibu-slo	Ibuprofen
Ibutad	Ibuprofen
Ibutid	Ibuprofen
Ibutop	Ibuprofen
ICI 35868	Propofol
ICI 66082	Atenolol
ICI 118630	Goserelin
Icipen	Penicillin V
Ifada	Famotidine
IFN- α ²	Interferon
IFN- α A	Interferon
Iktorivil	Clonazepam
Iletin II	Insulin
Ilosone (erythromycin estolate)	Erythromycin
Ilotycin	Erythromycin
Ilotycin Gluheptate (erythromycin glucoheptonate)	Erythromycin
Imex	Tetracycline

NAME

Imexim
 Imexim
 Imigran
 Imipem
 imipemide
 Imitrex
 Immukin (Gamma-1b)
 Immuneron (Cys-Tyr-Cys-interferon-gamma)
 Improntal
 Impugan
 Imuran
 Imurek
 Imurel
 Inabrin
 Inalone R
 Inalone O
 Incortin-H
 Indaflex
 Indamol
 Inderide
 Inderm
 Inestra
 Infectomycin
 Innovace
 Innozide
 Inostrat
 Inoven
 Instalac
 Intal
 Intensin
 interferon
 Interferon α A
 Interferon α 2b
 1-139 Interferon- γ
 Interferons- α
 Intracort (hydrocortisone 21-sodium succinate)
 Introcar
 Introl
 Intromycin
 Intron A (Alfa-2b)
 Introna (Alfa-2b)
 Inutral
 Investin
 Ipacef
 Ipamix
 ipratropium
 iproveratril
 Irtan
 isobamate
 5-isobutyl-5-allylbarbituric acid
p-isobutylhydratropic acid
 2-(4-isobutylphenyl)propionic acid

MONOGRAPH

Trimethoprim
 Sulfamethoxazole
 Sumatriptan
 Imipenem
 Imipenem
 Sumatriptan
 Interferon
 Interferon
 Piroxicam
 Furosemide
 Azathioprine
 Azathioprine
 Azathioprine
 Ibuprofen
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Hydrocortisone
 Indapamide
 Indapamide
 Hydrochlorothiazide
 Erythromycin
 Ethinyl Estradiol
 Amoxicillin
 Enalapril
 Enalapril
 Cromolyn
 Ibuprofen
 Trimethoprim
 Cromolyn
 Acetaminophen
 Interferon
 Interferon
 Interferon
 Interferon
 Interferon
 Hydrocortisone
 Nifedipine
 Cromolyn
 Neomycin
 Interferon
 Interferon
 Insulin
 Doxycycline
 Cefuroxime
 Indapamide
 Ipratropium Bromide
 Verapamil
 Cromolyn
 Carisoprodol
 Butalbital
 Ibuprofen
 Ibuprofen

NAME	MONOGRAPH
Isocillin	Penicillin V
Isoclor Expertorant C	Codeine
Isoclor Expectorant C	Guaifenesin
Iso-K	Ketoprofen
(±)-1-(isopropylamino)-3-[<i>p</i> -(β-methoxyethyl)phenoxy]-2-propanol	Metoprolol
isopropyl meprobamate	Carisoprodol
α-isopropyl-α-[(<i>N</i> -methyl- <i>N</i> -homoveratryl)-gamma-aminopropyl]-3,4-dimethoxyphenylacetoneitrile	Verapamil
<i>N</i> -isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate	Carisoprodol
8-isopropylnoratropine methobromide	Ipratropium Bromide
<i>N</i> -isopropylnoratropinium bromomethylate	Ipratropium Bromide
Isoptin	Verapamil
Isopto Hyoscine	Scopolamine
isotretinoin	Retinoic Acid
Isotrex (isotretinoin)	Retinoic Acid
Ispenoral	Penicillin V
Istin	Amlodipine
itobarbital	Butalbital
Itrin	Terazosin
Itrop	Ipratropium Bromide
Ituran	Nitrofurantoin
Ivadal	Zolpidem
Ivadantin	Nitrofurantoin
Ivaugan	Hydrochlorothiazide
Iwalexin	Cephalexin
Jatropur	Triamterene
Jen-Diril	Hydrochlorothiazide
Jenacyclin	Doxycycline
JM8	Carboplatin
Juvason	Prednisone
K 4024	Glipizide
K 3917	Temazepam
Kardiamed	Digoxin
Katlex	Furosemide
Keal	Sucralfate
Kefadin	Ceftazidime
Kefamin	Ceftazidime
Kefazim	Ceftazidime
Kefenid	Ketoprofen
Keflet	Cephalexin
Keflex	Cephalexin
Keforal	Cephalexin
Kefroxil	Cefadroxil
Keftab	Cephalexin
Kefurox	Cefuroxime
Kelfiprim	Trimethoprim
Kelp	Astemizole
Kenacort	Triamcinolone

NAME	MONOGRAPH
Kenacort Diacetate Syrup (triamcinolone diacetate)	Triamcinolone
Kenacort-A (triamcinolone acetonide)	Triamcinolone
Kenalog (triamcinolone acetonide)	Triamcinolone
Kenaquart (triamcinolone acetonide)	Triamcinolone
Kendall's compound F	Hydrocortisone
Kephina	Ketoprofen
Kepinol	Trimethoprim
Kepinol	Sulfamethoxazole
Kesint	Cefuroxime
Keteocort	Prednisone
Ketoderm	Ketoconazole
Ketoisdin	Ketoconazole
Ketopron	Ketoprofen
ketorolac	Ketorolac
Kevadon	Ketoprofen
Kinesed	Atropine
Kinesed	Scopolamine
Klacid	Clarithromycin
Klaricid	Clarithromycin
Klavikordal	Nitroglycerin
Klavocin	Clavulanic Acid
Klimicin	Clindamycin
Klonopin	Clonazepam
Kolikodal	Hydrocodone
Kolpolyn	Ethinyl Estradiol
Kombiquens	Ethinyl Estradiol
Kontexin	Phenylpropanolamine
Korbutone	Beclomethasone Dipropionate
Kordafen	Nifedipine
Korec	Quinapril
Korum	Acetaminophen
Kriplex	Diclofenac
KT-3777	Loracarbef
Kutrase	Atropine
Kwelcof	Guaifenesin
La III	Diazepam
Labazene	Valproic Acid
LaBID	Theophylline
Labophylline	Theophylline
Lacticare-HC	Hydrocortisone
Laevoxin	Levothyroxine
Lamdiol	Estradiol
Lamidon	Ibuprofen
Lamisil	Terbinafine
Lampsporin	Cefuroxime
Lamra	Diazepam
Lanacordin	Digoxin
Lanacort (hydrocortisone 21-acetate)	Hydrocortisone
Lanatilin	Digoxin
Landsen	Clonazepam
Lanicor	Digoxin

NAME

Lanoxin
 Laraflex
 Larapam
 Laratrim
 Laratrim
 Laridal
 Larixin
 Larocin
 Larotid
 Laroxyll
 Laser
 Lasilix
 Lasix
 Lasma
 Lastet
 Laticort (hydrocortisone 17-butyrate)
 Lauracycline
 Lauromicina (erythromycin estolate)
 LB-502
 Lebrufen
 Lecedil
 Ledercillin VK
 Ledercort Cream (triamcinolone acetonide)
 Ledercort D (triamcinolone acetonide)
 Lederglib
 Lederlon (triamcinolone hexacetonide)
 Lederspan (triamcinolone hexacetonide)
 Lehydán
 Lembrol
 Lenirit (hydrocortisone 21-acetate)
 Lenitral
 LenoxiCaps
 Lenoxin
 Lente
 Lente Iletin
 Lentizol
 Lentonitrina
 Lepitoin
 Lepitoin sodium
 Leponex
 Lepotex
 Leptilan
 Lergigan
 Lertus
 Lescol
 Letter
 Letusin
 6-D-leucine-9-(N-ethyl-L-prolinamide)-10-
 deglycinamidedeluteinizing hormone-releasing
 factor (pig)
 (D-Leu⁶)-des-Gly¹⁰-LH-RH-ethylamide
 Leuplin

MONOGRAPH

Digoxin
 Naproxen
 Piroxicam
 Trimethoprim
 Sulfamethoxazole
 Astemizole
 Cephalixin
 Amoxicillin
 Amoxicillin
 Amitriptyline
 Naproxen
 Furosemide
 Furosemide
 Theophylline
 Etoposide
 Hydrocortisone
 Tetracycline
 Erythromycin
 Furosemide
 Ibuprofen
 Famotidine
 Penicillin V
 Triamcinolone
 Triamcinolone
 Glyburide
 Triamcinolone
 Triamcinolone
 Phenytoin
 Diazepam
 Hydrocortisone
 Nitroglycerin
 Digoxin
 Digoxin
 Insulin
 Insulin
 Amitriptyline
 Nitroglycerin
 Phenytoin
 Phenytoin
 Clozapine
 Clozapine
 Valproic Acid
 Promethazine
 Ketoprofen
 Fluvastatin
 Levothyroxine
 Propoxyphene
 Leuprolide

 Leuprolide
 Leuprolide

NAME

leuprolide
 leuprorelin
 Levaxene
 Levaxol
 Levaxin
 Levium
 Levius
 Levlen
 Levlen
 levofloxacin
 levonorgestrel
 levopropoxyphene napsylate
 Levothroid
 Levothyrox
 levothyroxine
 Levsin
 Levsinex
 Lexibiotico
 Libanil
 Lidaform-HC (hydrocortisone acetate)
 Lidamantle-HC (hydrocortisone acetate)
 Lidaprim
 Lifeampil
 Limbitrol
 Linaris
 Linaris
 Linoral
 Liomycin
 Liponorm
 Lipostat
 Lipozid
 Liptan
 Lipur
 Liquamycin
 Liquiprin
 Lisacort
 Lisaglucon
 Lisino
 Litraderm (hydrocortisone acetate)
 Liviatin
 Lixidol
 Llonexina
 Lobufen
 Locoid (hydrocortisone 17-butyrate)
 Lodalès
 Lodine
 Loestrin
 Loestrin (norethindrone acetate)
 Loftan
 Logical
 Logynon
 Logynon

MONOGRAPH

Leuprolide
 Leuprolide
 Temazepam
 Temazepam
 Levothyroxine
 Diazepam
 Aspirin
 Ethinyl Estradiol
 Norgestrel
 Ofloxacin
 Norgestrel
 Propoxyphene
 Levothyroxine
 Levothyroxine
 Levothyroxine
 Atropine
 Atropine
 Cephalexin
 Glyburide
 Hydrocortisone
 Hydrocortisone
 Trimethoprim
 Ampicillin
 Amitriptyline
 Sulfamethoxazole
 Trimethoprim
 Ethinyl Estradiol
 Doxycycline
 Simvastatin
 Pravastatin
 Gemfibrozil
 Ibuprofen
 Gemfibrozil
 Tetracycline
 Acetaminophen
 Prednisone
 Glyburide
 Loratadine
 Hydrocortisone
 Doxycycline
 Ketorolac
 Cephalexin
 Ibuprofen
 Hydrocortisone
 Simvastatin
 Etodolac
 Ethinyl Estradiol
 Norethindrone
 Albuterol
 Valproic Acid
 Ethinyl Estradiol
 Norgestrel

NAME

Lomudal
 Lomudas
 Lomupren
 Lomusol
 Lomuspray
 Longasa
 Longdigox
 Lo/Ovral
 Lo-Ovral
 Lopid
 Lopirin
 Lopresor
 Lopressor
 Lopril
 Lorabid
 Lorasolid
 Lorax
 Loricin
 Lorsilan
 Losec
 Lotensin
 Lotrial
 Lotrimax
 Lotrimin
 Lotrisone
 Lotrisone (betamethasone 17,21-dipropionate)
 Lovalip
 Lowpston
 Lozol
 Lubricort
 Lucelan
 Lucrin
 Lupron
 Lupron Depot
 Lustral
 Luteolas
 Luteonorm
 Lutestral
 Luto-Metrodiol
 Lutorial
 LY061188
 LY-110140
 LY 139037
 LY 139381
 LY163892
 Lynoral
 Lyphocin
 Lyteca
 Mabertin
 Macasirool
 Macladin
 Mac-pac

MONOGRAPH

Cromolyn
 Cromolyn
 Cromolyn
 Cromolyn
 Cromolyn
 Aspirin
 Digoxin
 Norgestrel
 Ethinyl Estradiol
 Gemfibrozil
 Captopril
 Metoprolol
 Metoprolol
 Captopril
 Loracarbef
 Lorazepam
 Lorazepam
 Sulbactam
 Lorazepam
 Omeprazole
 Benazepril
 Enalapril
 Clotrimazole
 Clotrimazole
 Clotrimazole
 Betamethasone
 Lovastatin
 Furosemide
 Indapamide
 Hydrocortisone
 Buspirone
 Leuprolide
 Leuprolide
 Leuprolide
 Sertraline
 Ethynodiol Diacetate
 Ethynodiol Diacetate
 Ethinyl Estradiol
 Ethynodiol Diacetate
 Medroxyprogesterone Acetate
 Cephalixin
 Fluoxetine
 Nizatidine
 Ceftazidime
 Loracarbef
 Ethinyl Estradiol
 Vancomycin
 Acetaminophen
 Temazepam
 Furosemide
 Clarithromycin
 Nitrofurantoin

NAME

Macrobid
 Macrochantin
 Macrodiol
 Maderan
 Madlexin
 Maintasone
 Makrocef
 Malix
 Mamalexin
 Mamiesan
 Mandrozep
 Maninil
 Mantadil (hydrocortisone acetate)
 MAP
 Marcoeritrex (erythromycin estolate)
 Marevan
 Marisilan
 Marogen
 Marvelon 150/30
 Marvelon 150/30
 Maschitt
 Masdil
 Mastimyxin
 Maxitrol
 Maxitrol
 Maxivate (betamethasone 17,21-dipropionate)
 Maxzide
 Maxzide
 MD 6134
 MDL 9918
 Measurin
 Meberyt (erythromycin stearate)
 Mebroin
 Mecilex
 Medicort
 Mediletten
 Medipren
 Medoxim
 Medrate
 Medrol
 Medrol Stabisol (methylprednisolone 21-phosphate disodium salt)
 Medrone
 medroxyprogesterone
 Megacillin
 Melanate
 Melfax
 Menamin
 Menolyn
 Menorest
 Menova
 Menzol

MONOGRAPH

Nitrofurantoin
 Nitrofurantoin
 Estradiol
 Trimethoprim
 Cephalixin
 Hydrocortisone
 Cefotaxime
 Glyburide
 Cephalixin
 Dicyclomine
 Diazepam
 Glyburide
 Hydrocortisone
 Medroxyprogesterone Acetate
 Erythromycin
 Warfarin
 Ampicillin
 Epoetin
 Desogestrel
 Ethinyl Estradiol
 Hydrochlorothiazide
 Diltiazem
 Polymyxin
 Neomycin
 Polymyxin
 Betamethasone
 Hydrochlorothiazide
 Triamterene
 Warfarin
 Terfenadine
 Aspirin
 Erythromycin
 Phenytoin
 Cephalixin
 Hydrocortisone
 Tetracycline
 Ibuprofen
 Cefuroxime
 Methylprednisolone
 Methylprednisolone
 Methylprednisolone
 Methylprednisolone
 Methylprednisolone
 Medroxyprogesterone Acetate
 Penicillin V
 Tramadol
 Ranitidine
 Ketoprofen
 Ethinyl Estradiol
 Estradiol
 Ethinyl Estradiol
 Norethindrone

NAME

Mepergan
 Mephacyclin
 Mepral
 Mepred (methylprednisolone 21-acetate)
 Meprofen
 Merbentyl
 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline
 (*S*)-1-(3-methyl-1-mercapto-2-oxopropyl)-L-proline
 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline
 (2*S*)-1-(3-mercapto-2-methylpropionyl)-L-proline
 Mercilon
 Mercilon
 Mercodionone
 Meropenin
 Mespafin
 metacortandracin
 Metanite
 Metastab
 β-methasone
N-[4-(β-(2-methoxy-5-chlorobenzamido)ethyl)benzosulfonyl]-*N'*-cyclohexylurea
*N*¹-[4-[β-(2-methoxy-5-chlorobenzoylamino)ethyl]benzenesulfonyl]-*N*²-cyclohexylurea
 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol
 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole
 (*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid
 4-(6-methoxy-2-naphthalenyl)-2-butanone
 4-(6-methoxy-2-naphthyl)-butan-2-one
d-2-(6-methoxy-2-naphthyl)propionic acid
 3-(2-methoxyphenoxy)-1,2-propanediol
o-methoxyphenyl glyceryl ether
 6α-methyl-17α-acetoxypregesterone
N-[4-(6-methylamino-7-nitro-2-thia-5-aza-6-heptene-1-yl)-2-thiazolylmethyl]-*N,N*-dimethylamine
 5-(α-methylaminopropylidene)dibenzo[*a,d*]cyclohepta[1,4]diene
N-methyl-11-aza-10-deoxo-10-dihydroerythromycin A
 6α-methyl-11β,17α,21-triol-1,4-pregnadiene-3,20-dione
 2-methylbutanoic acid [1*S*-[1α(*R**),3α,7β,8β(2*S**,4*S**),8αβ]]-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)ethyl]-1-naphthalenyl ester

MONOGRAPH

Promethazine
 Tetracycline
 Omeprazole
 Methylprednisolone
 Ketoprofen
 Dicyclomine
 Captopril

 Captopril
 Captopril
 Captopril
 Desogestrel
 Ethinyl Estradiol
 Hydrocodone
 Penicillin V
 Doxycycline
 Prednisone
 Atropine
 Methylprednisolone
 Betamethasone
 Glyburide

 Glyburide

 Metoprolol

 Omeprazole

 Naproxen

 Nabumetone
 Nabumetone
 Naproxen
 Guaifenesin
 Guaifenesin
 Medroxyprogesterone Acetate
 Nizatidine

 Nortriptyline

 Azithromycin

 Methylprednisolone

 Lovastatin

NAME

methyl (2*S-trans*)-7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidiny]carbonyl]amino]-1-thio-L-threo- α -D-galactooctopyranoside
 6 α -methylcompactin
 methyl-diazepam
 6-*O*-methylerythromycin
 1-(methylethyl)carbamic acid 2-[[[(aminocarbonyl)oxy]methyl]-2-methylpentyl ester
 16 β -methyl-9 α -fluoro-delta¹-hydrocortisone
 16 β -methyl-9 α -fluoroprednisolone
 delta¹-6 α -methylhydrocortisone
 N¹-(5-methyl-3-isoxazolyl)sulfanilamide
 D-2-methyl-3-mercaptopropanoyl-L-proline
 α -methyl-4-(2-methylpropyl)benzeneacetic acid
 methylmorphine
trans-N-methyl-*N*-(1-naphthylmethyl)-6,6-dimethylhept-2-en-4-ynyl-1-amine
 6-(1-methyl-4-nitro-5-imidazolyl)mercaptopurine
 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine
 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide
N-methyloxazepam
 [2*S*-[2 α -(*E*),3 β ,4 β ,5 α [[2*R**,3*R**(1*R**,2*R**)]]]-9-[[3-methyl-1-oxo-4-trahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]nonanoic acid
 methylphenidan
 methylphenidate
 methyl phenidylacetate
 methyl α -phenyl- α -(2-piperidyl)acetate
 (\pm)-*N*-methyl-3-phenyl-3-[(α , α , α -trifluoro-*p*-tolyl)oxy]propylamine
 5-(2-methylpropyl)-5-(2-propenyl)-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione
 5-methyl-3-sulfanilamidoisoxazole
 (\pm)-*N*-methyl-gamma-[4-(trifluoromethyl)phenoxy]benzenepropanamine
N-methyl-3-(*p*-trifluoromethylphenoxy)-3-phenylpropylamine
 Meticorten
 Metodik
 Metoros
 Metracin
 Metrevet
 Metrisone
 Metrodiol
 Metropine
 Metrulen
 Meusicort

MONOGRAPH

Clindamycin

 Lovastatin
 Diazepam
 Clarithromycin
 Carisoprodol

 Betamethasone
 Betamethasone
 Methylprednisolone
 Sulfamethoxazole
 Captopril
 Ibuprofen
 Codeine
 Terbinafine

 Azathioprine
 Azathioprine

 Flutamide

 Temazepam
 Mupirocin

 Methylphenidate
 Methylphenidate
 Methylphenidate
 Methylphenidate
 Fluoxetine

 Butalbital

 Sulfamethoxazole
 Fluoxetine

 Fluoxetine

 Prednisone
 Astemizole
 Metoprolol
 Cimetidine
 Prednisone
 Methylprednisolone
 Ethynodiol Diacetate
 Atropine
 Ethynodiol Diacetate
 Hydrocortisone

NAME

Mevacor
 Mevalotin
 Mevinacor
 mevinolin
 Mevlor
 Micrainin
 Microgynon
 Microgynon
 Microlut
 Micronase
 Micronett
 Micronor
 Micronovum
 Microtrim
 Microtrim
 Microval
 midazolam
 Midol
 Midol 200
 Midoxin
 Miketorin
 Mildison
 Milligynon (norethindrone acetate)
 Millisrol
 Millithrol
 Milprem
 Miltax
 Milvane
 Mindiab
 Minetoin
 Mini-Pe
 Minidiab
 Miniluteolas
 Miniluteolas
 Minilyn
 "mini-pill"
 Minitran
 Minovlar (norethindrone acetate)
 Minulet
 Miocaina
 Miolisodal
 Mioril
 Miranax
 Mirfat
 MJ 9022-1
 MJF 11567-3
 MK-130
 MK-208
 MK-421
 MK-521
 MK-733
 MK-787

MONOGRAPH

Lovastatin
 Pravastatin
 Lovastatin
 Lovastatin
 Lovastatin
 Aspirin
 Ethinyl Estradiol
 Norgestrel
 Norgestrel
 Glyburide
 Norethindrone
 Norethindrone
 Norethindrone
 Trimethoprim
 Sulfamethoxazole
 Norgestrel
 Midazolam
 Acetaminophen
 Ibuprofen
 Doxycycline
 Amitriptyline
 Hydrocortisone
 Norethindrone
 Nitroglycerin
 Nitroglycerin
 Estrogens, Conjugated
 Ketoprofen
 Ethinyl Estradiol
 Glipizide
 Phenytoin
 Norethindrone
 Glipizide
 Ethinyl Estradiol
 Ethynodiol Diacetate
 Ethinyl Estradiol
 Norethindrone
 Nitroglycerin
 Norethindrone
 Ethinyl Estradiol
 Guaifenesin
 Carisoprodol
 Carisoprodol
 Naproxen
 Furosemide
 Buspirone
 Cefadroxil
 Cyclobenzaprine
 Famotidine
 Enalapril
 Lisinopril
 Simvastatin
 Imipenem

NAME

MK-791
 MK 803
 MK-906
 MK-950
 MM 14151
 MNPA
 Modacin
 Modicon
 Modicon
 Moduretic
 Mohrus
 Momentol
 Momentol
 Momentum
 mometasone
 monacolin K
 Mono-Attritin
 Mono-Baycuten
 Monodie
 Monomycin (erythromycin ethylsuccinate)
 Monopina
 Monotheamin
 Monotrim
 Monydrin
 Mopral
 Morepen
 Morosan
 Neurolytril
 morphine monomethyl ether
 morphine 3-methyl ether
 (—)-3-morpholino-4-(3-*tert*-butylamino)-2-hydroxypropoxy-1,2,5-thiadiazole
 Motiax
 Motovar (estradiol 3-benzoate)
 Motricit
 Motrin
 Motrin-A
 Moxal
 Moxaline
 Muclox
 Mucorama
 Mudrane GG Elixir
 MY-301
 Myacine
 Myacyne
 Mycelex
 Mycelex-G
 Mycifradin
 Myciguent
 Mycofug
 Mycolog II (triamcinolone acetone)
 Mycosporin

MONOGRAPH

Cilastatin
 Lovastatin
 Finasteride
 Timolol
 Clavulanic Acid
 Naproxen
 Cefprozil
 Ethinyl Estradiol
 Norethindrone
 Hydrochlorothiazide
 Ketoprofen
 Sulfamethoxazole
 Trimethoprim
 Acetaminophen
 Mometasone Furoate
 Lovastatin
 Ibuprofen
 Clotrimazole
 Ethinyl Estradiol
 Erythromycin
 Amlodipine
 Theophylline
 Trimethoprim
 Phenylpropanolamine
 Omeprazole
 Ampicillin
 Diazepam
 Diazepam
 Codeine
 Codeine
 Timolol
 Famotidine
 Estradiol
 Ibuprofen
 Ibuprofen
 Ibuprofen
 Amoxicillin
 Amoxicillin
 Famotidine
 Phenylpropanolamine
 Theophylline
 Guaifenesin
 Neomycin
 Neomycin
 Clotrimazole
 Clotrimazole
 Neomycin
 Neomycin
 Clotrimazole
 Triamcinolone
 Clotrimazole

NAME

Myco-Triacet II (triamcinolone acetonide)
 mydriatin
 Mydriatine
 Mylproin
 Mynosedin
 Myocaine
 Myocord
 Myoglycerin
 Myoscain
 Mysteclin-F
 Mytrex (triamcinolone acetonide)
 Nabuser
 Nadigest
 Naixan
 Nalcrom
 Nalcron
 Naldecon
 Naldecon-CX
 Naldecon-CX
 Naldecon-DX
 Naldecon-EX
 Naldegescic
 Naldetuss
 Naldetuss
 Nanormon
 Napacetin
 Napren
 Naprinol
 Naprium
 Naprius
 Naprosine
 Naprosyn
 Naprosyne
 Naprux
 Narilet
 Nasacort (triamcinolone acetonide)
 Nasalcrom
 Nasmil
 Natrilix
 Naxen
 Naxidine
 Naxy
 Neamoxyl
 Nebralin
 Nebs
 Nefrix
 Neobrettin
 Neo-Codema
 Neocon 1/35
 Neocon 1/35
 Neo-Cort-Dome
 Neo-Cort-Dome

MONOGRAPH

Triamcinolone
 Phenylpropanolamine
 Phenylpropanolamine
 Valproic Acid
 Ibuprofen
 Guaifenesin
 Atenolol
 Nitroglycerin
 Guaifenesin
 Tetracycline
 Triamcinolone
 Nabumetone
 Medroxyprogesterone Acetate
 Naproxen
 Cromolyn
 Cromolyn
 Phenylpropanolamine
 Codeine
 Guaifenesin
 Guaifenesin
 Guaifenesin
 Acetaminophen
 Acetaminophen
 Phenylpropanolamine
 Somatropin
 Ibuprofen
 Naproxen
 Acetaminophen
 Naproxen
 Naproxen
 Naproxen
 Naproxen
 Naproxen
 Naproxen
 Naproxen
 Naproxen
 Naproxen
 Ipratropium Bromide
 Triamcinolone
 Cromolyn
 Cromolyn
 Indapamide
 Naproxen
 Nizatidine
 Clarithromycin
 Amoxicillin
 Terfenadine
 Acetaminophen
 Hydrochlorothiazide
 Neomycin
 Hydrochlorothiazide
 Ethinyl Estradiol
 Norethindrone
 Hydrocortisone
 Neomycin

NAME	MONOGRAPH
Nitrocontin	Nitroglycerin
Nitroderm TTS	Nitroglycerin
Nitrodisc	Nitroglycerin
Nitro-Dur	Nitroglycerin
Nitrofortin	Nitroglycerin
1-[[<i>(5-nitro-2-furanyl)methylene</i> amino]-2,4-imidazolidinedione	Nitrofurantoin
1-(5-nitro-2-furfurylideneamino)hydantoin	Nitrofurantoin
<i>N</i> -(5-nitro-2-furfurylidene)-1-aminohydantoin	Nitrofurantoin
Nitrogard	Nitroglycerin
Nitro-Gesanit	Nitroglycerin
Nitroglin	Nitroglycerin
nitroglycerol	Nitroglycerin
Nitroglyn	Nitroglycerin
Nitrol Creme	Nitroglycerin
Nitrolan	Nitroglycerin
Nitrolande	Nitroglycerin
Nitrolar	Nitroglycerin
Nitrolent	Nitroglycerin
Nitrolingual	Nitroglycerin
Nitrolingual Spray	Nitroglycerin
Nitro Mack	Nitroglycerin
Nitromel	Nitroglycerin
Nitromex	Nitroglycerin
Nitronal	Nitroglycerin
Nitronal Aqueous	Nitroglycerin
Nitronet	Nitroglycerin
Nitrong	Nitroglycerin
Nitro-Pflaster-ratiopharm	Nitroglycerin
4-(2'-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine	Nifedipine
NitroPRN	Nitroglycerin
Nitrorectal	Nitroglycerin
Nitroretard	Nitroglycerin
Nitrosigma	Nitroglycerin
Nitrospan	Nitroglycerin
Nitrostat	Nitroglycerin
4'-nitro-3'-trifluoromethylisobutyranilide	Flutamide
Nitrozell-retard	Nitroglycerin
Nivemycin	Neomycin
Nivocilin	Doxycycline
Nizax	Nizatidine
Nizaxid	Nizatidine
Nizoral	Ketoconazole
NK 171	Etoposide
Noan	Diazepam
Nobedon	Acetaminophen
Nobfelon	Ibuprofen
Nobfen	Ibuprofen
Nobgen	Ibuprofen
Noctone	Ranitidine
Nopil	Sulfamethoxazole

NAME

Nopil
 Noracyclin
 Noralutin
 Noranat
 Norcolut
 Nordette
 Nordette
 Nordicort (hydrocortisone 21-sodium succinate)
 Norditropin
 Nordox
dl-norephedrine hydrochloride
 Norethin 1/35 E
 Norethin 1/50 M
 19-norethisterone
 Norethrin 1/35 E
 19-nor-17 α -ethynylandrosten-17 β -ol-3-one
 19-nor-17 α -ethynyl-17 β -hydroxy-4-androsten-3-one
 19-nor-17 α -ethynyltestosterone
 Norgan
 Norgesic
 Norgeston
 Noriday
 Noridyl
 Norimin
 Norimin
 Norinyl 1+35
 Norinyl 1+35
 Norinyl
 Norinyl-1
 Noristerat (norethindrone enanthate)
 Noritren
 Norlestrin
 Norlestrin (norethindrone acetate)
 Norlutate (norethindrone acetate)
 Norluten
 Norlutin
 Norlutin-A (norethindrone acetate)
 Norluton
 Norma-oestren
 Normison
 Normothen
 Norodine
 Norplant
 (17 α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol
 norpregneninolone
 (3 β ,17 α)-19-norpregn-4-en-20-yne-3,17-diol diacetate
 Nor-QD
 Norquentiel
 Norquentiel

MONOGRAPH

Trimethoprim
 Ethinyl Estradiol
 Norethindrone
 Indapamide
 Norethindrone
 Ethinyl Estradiol
 Norgestrel
 Hydrocortisone
 Somatropin
 Doxycycline
 Phenylpropanolamine
 Norethindrone
 Norethindrone
 Norethindrone
 Ethinyl Estradiol
 Norethindrone
 Norethindrone
 Norethindrone
 Hydrocodone
 Aspirin
 Norgestrel
 Norethindrone
 Triamterene
 Ethinyl Estradiol
 Norethindrone
 Ethinyl Estradiol
 Norethindrone
 Norethindrone
 Norethindrone
 Norethindrone
 Nortriptyline
 Ethinyl Estradiol
 Norethindrone
 Norethindrone
 Norethindrone
 Norethindrone
 Norethindrone
 Ethinyl Estradiol
 Temazepam
 Doxazosin
 Trimethoprim
 Norgestrel
 Ethinyl Estradiol
 Norethindrone
 Ethynodiol Diacetate
 Norethindrone
 Ethinyl Estradiol
 Norethindrone

NAME

Nortrilen
 nortriptyline
 Norzepine
 Notul
 Novadox
 Noval
 Novapirina
 Novasam
 Novatec
 Novestrol
 Novocef
 Novodigal
 Novogent N
 Novolin
 Novo-Nastizol A
 Novrad
 NPH
 NPH Iletin
 NSC-9564
 NSC-9566 (estradiol 3-benzoate)
 NSC-9895
 NSC-10023
 NSC-10483
 NSC-10973
 NSC-17590 (estradiol 17-valerate)
 NSC-19043
 NSC-19987
 NSC-20293
 NSC-26386
 NSC-39470
 NSC-77518
 NSC-77625
 NSC-106568
 NSC-122758 (tretinoin)
 NSC-125973
 NSC-129224
 NSC-141540
 NSC-241240
 NSC-339140 (Alfa-n1)
 N-Toin
 Nucofed
 Nucofed Expectorant
 Nuelin
 Nulcerin
 Numidan
 Nuprin
 Nurison
 Nurofen
 Nutracort
 Nutropin
 Nuvacon
 Nuvapen

MONOGRAPH

Nortriptyline
 Nortriptyline
 Nortriptyline
 Cimetidine
 Doxycycline
 Ethinyl Estradiol
 Diclofenac
 Diazepam
 Lisinopril
 Ethinyl Estradiol
 Cefuroxime
 Digoxin
 Ibuprofen
 Insulin
 Astemizole
 Propoxyphene
 Insulin
 Insulin
 Norethindrone
 Estradiol
 Estradiol
 Prednisone
 Hydrocortisone
 Ethinyl Estradiol
 Estradiol
 Oxycodone
 Methylprednisolone
 Estradiol
 Medroxyprogesterone Acetate
 Betamethasone
 Diazepam
 Triamterene
 Trimethoprim
 Retinoic Acid
 Paclitaxel
 Adiphenine
 Etoposide
 Carboplatin
 Interferon
 Nitrofurantoin
 Codeine
 Guaifenesin
 Theophylline
 Famotidine
 Naproxen
 Ibuprofen
 Prednisone
 Ibuprofen
 Hydrocortisone
 Somatropin
 Ethinyl Estradiol
 Ampicillin

NAME

Nycopren
 Nysconitrine
 Nystaform-HC
 Obestat
 Odemase
 Oedemex
 Oestergon
 oestradiol
 Oestroform (estradiol 3-benzoate)
 Oestrogel
 Oflocet
 Oflocin
 Oflox
 ofloxacin
 Ohlexin
 Oliprevin
 Olivin
 Omcilon
 Omcilon-A (triamcinolone acetonide)
 Omegamycin
 Omepal
 Omeprazen
 Omnipen
 Omnipen-N
 Omsat
 Omsat
 ondansetron
 Ophthocort
 Ophthocort
 Opticrom
 Opticron
 Optisulin long
 Optium
 Opturem
 Oracef
 Oracéfal
 Oracil-VK
 Oracilline
 Oracocin
 Oracon
 Oradiol
 Orafuran
 Oragest
 Orapen V-K
 Oraprim
 Oraprim
 Orasone
 Oratren
 Oraxim (cefuroxime axetil)
 Orbicilina
 Oresol
 Oreson

MONOGRAPH

Naproxen
 Nitroglycerin
 Hydrocortisone
 Phenylpropanolamine
 Furosemide
 Furosemide
 Estradiol
 Estradiol
 Estradiol
 Estradiol
 Ofloxacin
 Ofloxacin
 Ofloxacin
 Ofloxacin
 Ofloxacin
 Cephalexin
 Pravastatin
 Enalapril
 Triamcinolone
 Triamcinolone
 Tetracycline
 Omeprazole
 Omeprazole
 Ampicillin
 Ampicillin
 Trimethoprim
 Sulfamethoxazole
 Ondansetron
 Hydrocortisone
 Polymyxin
 Cromolyn
 Cromolyn
 Insulin
 Amoxicillin
 Ibuprofen
 Cephalexin
 Cefadroxil
 Penicillin V
 Penicillin V
 Cephalexin
 Ethinyl Estradiol
 Ethinyl Estradiol
 Nitrofurantoin
 Medroxyprogesterone Acetate
 Penicillin V
 Trimethoprim
 Sulfamethoxazole
 Prednisone
 Penicillin V
 Cefuroxime
 Ampicillin
 Guaifenesin
 Guaifenesin

NAME

Orestralyn
 Oretic
 Orfiril
 Org-2969
 Orgasuline
 Orifungal M
 Orion
 Orix
 Ornade
 Ornex
 Orocillin
 Oroken
 Oroxine
 Ortensan
 Ortho-Cept
 Ortho-Cept
 Ortho-Cyclen
 Ortho-Novin 1/50
 Ortho-Novum
 Ortho-Novum 1/35
 Ortho-Novum 1/35
 Ortho-Novum 1/50
 Ortho-Novum
 Ortho-Novum 7/7/7
 Ortho-Novum 7/7/7
 Orthoxycol
 Ortisporina
 Orudis
 Orugesic
 Oruvail
 Oscorel
 Ospamox
 Oспен
 Ospeneff
 Ostro-Primolut
 Otalgine (neomycin undecylenate)
 Otalgine
 Otic-Neo-Cort-Dome
 Otic-Neo-Cort-Dome
 Otobiotic
 Otobiotic
 Otocort
 Otocort
 Otocort
 Ovahormon
 Ovahormon Benzoate (estradiol 3-benzoate)
 Ovahormon Depot (estradiol dipropionate)
 Ovanon
 Ovaras
 Ovasterol
 Ovasterol-B (estradiol 3-benzoate)
 Ovcon

MONOGRAPH

Ethinyl Estradiol
 Hydrochlorothiazide
 Valproic Acid
 Desogestrel
 Insulin
 Ketoconazole
 Triamcinolone
 Nifedipine
 Phenylpropanolamine
 Acetaminophen
 Penicillin V
 Cefixime
 Levothyroxine
 Acetaminophen
 Desogestrel
 Ethinyl Estradiol
 Ethinyl Estradiol
 Norethindrone
 Ethinyl Estradiol
 Ethinyl Estradiol
 Norethindrone
 Norethindrone
 Norethindrone
 Norethindrone
 Ethinyl Estradiol
 Hydrocodone
 Cephalixin
 Ketoprofen
 Ketoprofen
 Ketoprofen
 Ketoprofen
 Amoxicillin
 Penicillin V
 Penicillin V
 Norethindrone
 Neomycin
 Hydrocortisone
 Neomycin
 Hydrocortisone
 Hydrocortisone
 Polymyxin
 Neomycin
 Hydrocortisone
 Polymyxin
 Estradiol
 Estradiol
 Estradiol
 Ethinyl Estradiol
 Ethynodiol Diacetate
 Estradiol
 Estradiol
 Ethinyl Estradiol

NAME

Ovcon
 Ovex B (estradiol 3-benzoate)
 Ovin
 Oviol
 Oviol
 Ovocyclin
 Ovocyclin Benzoate (estradiol 3-benzoate)
 Ovocyclin dipropionate (estradiol dipropionate)
 Ovocyclin M (estradiol 3-benzoate)
 Ovocyclin-MB (estradiol 3-benzoate)
 Ovocyclin-P (estradiol dipropionate)
 Ovocyclin
 Ovoresta
 Ovral
 Ovral
 Ovran
 Ovran
 Ovranette
 Ovranette
 Ovrette
 Ovulen
 Ovysmen
 Ovysmen
 Oxaldin
 Oxapro
 Oxcord
 Oxikon
 1-(5-oxohexyl)-3,7-dimethylxanthine
 1-(5-oxohexyl)theobromine
 oxpentifylline
 Oxycon
 oxydiazepam
 Oxyphyllin
 P 50
 rt-PA
 t-PA
 Pabracort (hydrocortisone acetate)
 P-A-C Analgesic Tablets
 Pacemol
 Paceum
 Pacitran
 Paediathrocin (erythromycin ethylsuccinate)
 Paidomal
 Paldesic
 Paldomycin
 Palonyl
 Pamelor
 Pamocil
 Panacef
 Panadol
 Panaleve
 Panalog (triamcinolone acetone)

MONOGRAPH

Norethindrone
 Estradiol
 Ethinyl Estradiol
 Desogestrel
 Ethinyl Estradiol
 Estradiol
 Estradiol
 Estradiol
 Estradiol
 Estradiol
 Estradiol
 Ethinyl Estradiol
 Ethinyl Estradiol
 Norgestrel
 Ethinyl Estradiol
 Norgestrel
 Ethinyl Estradiol
 Norgestrel
 Norgestrel
 Norgestrel
 Ethynodiol Diacetate
 Ethinyl Estradiol
 Norethindrone
 Ofloxacin
 Oxaprozin
 Nifedipine
 Oxycodone
 Pentoxifylline
 Pentoxifylline
 Pentoxifylline
 Oxycodone
 Temazepam
 Theophylline
 Ampicillin
 Alteplase
 Alteplase
 Hydrocortisone
 Aspirin
 Acetaminophen
 Diazepam
 Diazepam
 Erythromycin
 Theophylline
 Acetaminophen
 Doxycycline
 Ethinyl Estradiol
 Nortriptyline
 Amoxicillin
 Cefaclor
 Acetaminophen
 Acetaminophen
 Triamcinolone

NAME	MONOGRAPH
Panasorb	Acetaminophen
Pancodine	Oxycodone
Percocet	Oxycodone
Pandel (hydrocortisone buteprate)	Hydrocortisone
Panets	Acetaminophen
Panex	Acetaminophen
Panfungol	Ketoconazole
Panlomyc	Terconazole
Panmycin	Tetracycline
Panmycin P	Tetracycline
Panodil	Acetaminophen
Panofen	Acetaminophen
Panophylline	Theophylline
Panoral	Cefaclor
Pantomicina (erythromycin stearate)	Erythromycin
Panurin	Hydrochlorothiazide
Panwarfin	Warfarin
Panzid	Ceftazidime
paracetamol	Acetaminophen
Paracort	Prednisone
Paralatin	Carboplatin
Paralergin	Astemizole
Paraspen	Acetaminophen
Parbetan (betamethasone 17-benzoate)	Betamethasone
Parelan	Acetaminophen
Parfuran	Nitrofurantoin
Parizac	Omeprazole
Parlodel	Bromocriptine
Parmol	Acetaminophen
Partrex	Tetracycline
Pasetocin	Amoxicillin
Pasolind	Acetaminophen
Pasolind N	Acetaminophen
Patrovina	Adiphenine
Paxate	Diazepam
Paxel	Diazepam
Paxofen	Ibuprofen
PCE	Erythromycin
PD 109452-2	Quinapril
Pediamycin (erythromycin ethylsuccinate)	Erythromycin
Pediaprofen	Ibuprofen
Pediazole (erythromycin ethylsuccinate)	Erythromycin
Pediotic Suspension	Neomycin
Pediotic Suspension	Hydrocortisone
Pediotic Suspension	Polymyxin
Pedipen	Penicillin V
Pedisafe	Clotrimazole
Pelanin benzoate (estradiol 3-benzoate)	Estradiol
Pelanin Depot (estradiol 17-valerate)	Estradiol
PELS (erythromycin estolate)	Erythromycin
pemophyllin	Theophylline
Pen A	Ampicillin

NAME

Penagen
 Penamox
 Pen A/N
 Penapar VK
 Penavlon V
 Penbristol
 Penbritin
 Penbritin-S
 Penbrock
 Pencompren
 Penecort
 Penialmen
 Penicals
 penicillanic acid sulfone
 penicillanic acid 1,1-dioxide
 penicillin phenoxymethyl
 Pénicline
 Penimox
 Penntuss
 Pen-Oral
 Penstabil
 Pensyn
 Pentrex
 Pentrexyl
 Pen-Vee
 Pen-Vee K
 Penvikal
 Pepcid
 Pepcidina
 Pepcidine
 Pepdine
 Pepdul
 Peptan
 Pepticum
 Peptol
 Percocet
 Percodan
 Percodan
 Percodan Demi
 Percogesic
 Percutol
 Perdorm
 Perglotal
 Perlatanol
 Perlinganit
 Perlutex
 Perovex
 Persistin
 Pfizer-E (erythromycin stearate)
 Pfizerpen VK
 Phaeva
 Phenaphen

MONOGRAPH

Penicillin V
 Amoxicillin
 Ampicillin
 Penicillin V
 Penicillin V
 Ampicillin
 Ampicillin
 Ampicillin
 Ampicillin
 Penicillin V
 Hydrocortisone
 Ampicillin
 Penicillin V
 Sulbactam
 Sulbactam
 Penicillin V
 Ampicillin
 Amoxicillin
 Codeine
 Penicillin V
 Ampicillin
 Ampicillin
 Ampicillin
 Ampicillin
 Penicillin V
 Penicillin V
 Penicillin V
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Famotidine
 Omeprazole
 Cimetidine
 Acetaminophen
 Aspirin
 Oxycodone
 Oxycodone
 Codeine
 Nitroglycerin
 Temazepam
 Nitroglycerin
 Estradiol
 Nitroglycerin
 Medroxyprogesterone Acetate
 Ethinyl Estradiol
 Aspirin
 Erythromycin
 Penicillin V
 Ethinyl Estradiol
 Acetaminophen

NAME

Phencen
 Phenergan
 Phenhydan
 Phenidylate
 Phenopenicillin
 6-phenoxyacetamidopenicillamic acid
 phenoxymethylpenicillin
 phenoxymethylpenicillinic acid
 Phensal
 3- α -phenyl- β -acetyloethyl-4-hydroxycoumarin
 α -phenylbenzeneacetic acid 2-
 (diethylamino)ethyl ester hydrochloride
 α -phenyl-2-piperidineacetic acid methyl ester
 α -phenyl- α -(2-piperidyl)acetic acid methyl ester
 phenylpropanolamine
 6-phenyl-2,4,7-pteridinetriamine
 6-phenyl-2,4,7-triaminopteridine
 Phylol
 phyone
 Physpan
 Pidilat
 Pimavacort
 piroxene
 Piramox
 Pirkam
 Piroflex
 pituitary growth hormone
 Plancol (hydrocortisone 17-butyrate)
 Planovin
 Planum
 plasminogen activator (human tissue-type 2-
 chain form protein moiety)
 Platet
 PMB-200
 PMB-400
 P-Mega-Tabliten
 Polcortolon (triamcinolone diacetate)
 Polixima
 Polycillin
 Polycillin-N
 Polycycline
 Polyferon (Cys-Tyr-Cys-interferon-gamma)
 Polyflex
 Polymox
 polymyxin B
 Poly-PRB
 Poly-Pred
 Poly-Pred
 Polysporin
 Polytrim
 Polytrim
 Ponecil

MONOGRAPH

Promethazine
 Promethazine
 Phenytoin
 Methylphenidate
 Penicillin V
 Penicillin V
 Penicillin V
 Penicillin V
 Aspirin
 Warfarin
 Adiphenine

 Methylphenidate
 Methylphenidate
 Phenylpropanolamine
 Triamterene
 Triamterene
 Somatropin
 Somatropin
 Theophylline
 Nifedipine
 Neomycin
 Naproxen
 Amoxicillin
 Piroxicam
 Piroxicam
 Somatropin
 Hydrocortisone
 Ethinyl Estradiol
 Temazepam
 Alteplase

 Aspirin
 Estrogens, Conjugated
 Estrogens, Conjugated
 Penicillin V
 Triamcinolone
 Cefuroxime
 Ampicillin
 Ampicillin
 Tetracycline
 Interferon
 Ampicillin
 Amoxicillin
 Polymyxin
 Ampicillin
 Neomycin
 Polymyxin
 Polymyxin
 Trimethoprim
 Polymyxin
 Ampicillin

NAME

Poviral
 Praecigucon
 Pramace
 Pranoxen
 Pravachol
 Pravaselect
 pravastatin
 Pravidel
 Precortal
 Prednilonga
 1,4-pregnadiene-17 α ,21-diol-3,11,20-trione
 4-pregnene-11 β ,17 α ,21 triol-3,20-dione
 Prelis
 Premarin
 Prenormine
 Prepcort
 Prepulsid
 Pres
 Pressural
 Pretor
 Prexan
 Prilosec
 Primatene Tablets
 Primaxin
 Primaxin
 Primeral
 Primofenac
 Primofol (estradiol 17-valerate)
 Primogyn
 Primogyn B (estradiol 3-benzoate)
 Primogyn C
 Primogyn I (estradiol 3-benzoate)
 Primogyn M
 Primolut N
 Primolut-Nor (norethindrone acetate)
 Primosiston (norethindrone acetate)
 Princillin
 Principen
 Principen/N
 Prinil
 Priinivil
 Prinzide
 Prinzide
 proazamine
 Procardia
 Procef
 Procrit
 Proctocort
 Proctofoam-HC (hydrortisone acetate)
 Procyclomin
 Prodasone
 Pro Dorm

MONOGRAPH

Acyclovir
 Glyburide
 Ramipril
 Naproxen
 Pravastatin
 Pravastatin
 Pravastatin
 Bromocriptine
 Prednisone
 Prednisone
 Prednisone
 Hydrocortisone
 Metoprolol
 Estrogens, Conjugated
 Atenolol
 Hydrocortisone
 Cisapride
 Enalapril
 Indapamide
 Cefotaxime
 Naproxen
 Omeprazole
 Theophylline
 Cilastatin
 Imipenem
 Naproxen
 Diclofenac
 Estradiol
 Ethinyl Estradiol
 Estradiol
 Ethinyl Estradiol
 Estradiol
 Ethinyl Estradiol
 Norethindrone
 Norethindrone
 Norethindrone
 Ampicillin
 Ampicillin
 Ampicillin
 Lisinopril
 Lisinopril
 Lisinopril
 Hydrochlorothiazide
 Promethazine
 Nifedipine
 Cefprozil
 Epoetin
 Hydrocortisone
 Hydrocortisone
 Dicyclomine
 Medroxyprogesterone Acetate
 Lorazepam

NAME

Profemin
 Profenid
 Proflax
 Proflex
 Profoliol
 Profoliol B
 Progylut
 Progynon
 Progynon-B (estradiol 3-benzoate)
 Progynon Benzoate (estradiol 3-benzoate)
 Progynon C
 Progynon Depot (estradiol 17-valerate)
 Progynon-DH
 Progynon-DP (estradiol dipropionate)
 Progynon M
 Progynova (estradiol 17-valerate)
 proheptatriene
 Proladone
 Proloprim
 Prolysis
 Promacortine
 Promantine
 promethazine
 Promethegan
 Promine
 Pronison
 Prontalgin
 Propacet
 Propacet
 Propaderm
 Propadrine hydrochloride
 Pro-Pam
 propanetriol trinitrate 1,2,3-
 Prophenatin
 Propiocrine Enfant (erythromycin estolate)
 Propiocrine
 Propox
 Propoxychel
 propoxyphene
d-propoxyphene
 Propulsid
 Propulsin
 di-*n*-propylacetic acid
 2-propylpentanoic acid
 2-propylvaleric acid
 Prorex
 Proscar
 Prostap
 Prostide
 Protef
 Protef (hydrortisone acetate)
 Prothazine

MONOGRAPH

Furosemide
 Ketoprofen
 Timolol
 Ibuprofen
 Estradiol
 Estradiol
 Ethinyl Estradiol
 Estradiol
 Estradiol
 Estradiol
 Ethinyl Estradiol
 Estradiol
 Estradiol
 Estradiol
 Ethinyl Estradiol
 Estradiol
 Cyclobenzaprine
 Oxycodone
 Trimethoprim
 Alteplase
 Methylprednisolone
 Promethazine
 Promethazine
 Promethazine
 Promethazine
 Prednisone
 Ibuprofen
 Acetaminophen
 Propoxyphene
 Beclomethasone Dipropionate
 Phenylpropanolamine
 Diazepam
 Nitroglycerin
 Diclofenac
 Erythromycin
 Erythromycin
 Propoxyphene
 Propoxyphene
 Propoxyphene
 Propoxyphene
 Propoxyphene
 Cisapride
 Cisapride
 Valproic Acid
 Valproic Acid
 Valproic Acid
 Promethazine
 Finasteride
 Leuprolide
 Finasteride
 Neomycin
 Hydrocortisone
 Promethazine

NAME

Prothromadin
 Pro-Vent
 Proventil
 Provera
 Provest
 Provest
 Provigan
 Proxagesic
 Proxen
 Proxine
 Prozac
trans-pseudomonic acid
 pseudomonic acid A
 Psicopax
 Psorion (betamethasone 17,21-dipropionate)
 Psychostyl
 pterofen
 pterophene
 PulmiDur
 Pulmo-Timelets
 Punktyl
 Purocyclina
 PV Tussin
 PV Tussin Tablet
 PVK
 Pylorid
 Pyridium Plus
 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione
 N-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-1,1-cyclopentanediacetamide
 QI Damp
 Q-Pam
 Quadracyclin
 Quait
 Quark
 Quasar
 Quatrex
 Quibron
 Quibron
 Quiet World
 quinapril
 Quinazil
 R 41400
 R 42470
 R 43512
 R 51619
 Racet
 Ramace
 Ramodar
 Raniben
 Ranidil

MONOGRAPH

Warfarin
 Theophylline
 Albuterol
 Medroxyprogesterone Acetate
 Medroxyprogesterone Acetate
 Ethinyl Estradiol
 Promethazine
 Propoxyphene
 Naproxen
 Naproxen
 Fluoxetine
 Mupirocin
 Mupirocin
 Lorazepam
 Betamethasone
 Nortriptyline
 Triamterene
 Triamterene
 Theophylline
 Theophylline
 Lorazepam
 Tetracycline
 Hydrocodone
 Guaifenesin
 Penicillin V
 Ranitidine
 Atropine
 Buspirone

 Buspirone

 Ampicillin
 Diazepam
 Tetracycline
 Lorazepam
 Ramipril
 Verapamil
 Tetracycline
 Guaifenesin
 Theophylline
 Acetaminophen
 Quinapril
 Quinapril
 Ketoconazole
 Terconazole
 Astemizole
 Cisapride
 Hydrocortisone
 Ramipril
 Etodolac
 Ranitidine
 Ranitidine

NAME

Raniplex
 Rapinovet
 Raylina
 RD 13621
 Rebugen
 Recidol
 Recthormone Oestradiol (estradiol 3-benzoate)
 Rectodelt
 Rectoid
 Redicilin
 Redomex
 Refkas (erythromycin ethylsuccinate)
 Reichstein's substance M
 Rela
 Relafen
 Relanium
 Relasom
 Relaxil G
 Relifen
 Relifex
 Remestan
 Remicyclin
 Reminitrol
 Remsed
 Renacor
 Renacor
 Reneuron
 Renitec
 Reniten
 Renivace
 Rentylin
 Reorganin
 Respbid
 Respenyl
 Respicort (triamcinolone acetonide)
 Restoril
 Resyl
 Retcin
 Retens
 Retin-A (tretinoin)
 Retolen
 retrocortine
 Retrovir
 Reudene
 Reuxen
 RG 83606
 Rhinex D-Lay
 Rhinex D-Lay tablets
 Rhodine
 Rhumalgan
 Riacen
 Rimazole

MONOGRAPH

Ranitidine
 Propofol
 Amoxicillin
 Ibuprofen
 Ibuprofen
 Ibuprofen
 Estradiol
 Prednisone
 Hydrocortisone
 Ampicillin
 Amitriptyline
 Erythromycin
 Hydrocortisone
 Carisoprodol
 Nabumetone
 Diazepam
 Carisoprodol
 Guaifenesin
 Nabumetone
 Nabumetone
 Temazepam
 Tetracycline
 Nitroglycerin
 Promethazine
 Enalapril
 Lisinopril
 Fluoxetine
 Enalapril
 Enalapril
 Enalapril
 Pentoxifylline
 Guaifenesin
 Theophylline
 Guaifenesin
 Triamcinolone
 Temazepam
 Guaifenesin
 Erythromycin
 Doxycycline
 Retinoic Acid
 Astemizole
 Prednisone
 Zidovudine
 Piroxicam
 Naproxen
 Diltiazem
 Acetaminophen
 Phenylpropanolamine
 Aspirin
 Diclofenac
 Piroxicam
 Clotrimazole

NAME

Rinatec
Rinderon-DP (betamethasone 17,21-
dipropionate)
Rinesal
Rineton (triamcinolone acetonide)
Rino-Clenil
Riocyclin
Risamal
Ritalin
Ritalin Hydrochloride
Rivotril
RMI 9918
Ro 4-1575
Ro 4-1577
Ro 4-2130
Ro 4-3780 (isotretinoin)
Ro 5-2807
Ro 5-4023
Ro 5-5345
Ro-13-9904
Ro-13-9904/001
Ro 21-3981/001
Ro 21-3981/003
Ro 22-8181 (Alfa-2a)
Roaccutane (isotretinoin)
Ro-Ampen
Robamox
Robaxisal
Robicillin VK
Robitet
Robitussin
Rocefin
Rocephin(e)
Ro-Cycline
Rodex
Roferon-A (Alfa-2a)
Ro-Hydrazide
Roidenin
Roldiol
Rona-Phyllin
Ronaxan
Rosampline
Rosemide
Rougoxin
Roxicam
Roxiden
Roximycin
Roxomicina (erythromycin estolate)
RP 3277
RP 3389
RP 9715
RP 19583

MONOGRAPH

Ipratropium Bromide
Betamethasone
Cephalexin
Triamcinolone
Beclomethasone Dipropionate
Tetracycline
Cisapride
Methylphenidate
Methylphenidate
Clonazepam
Terfenadine
Amitriptyline
Cyclobenzaprine
Sulfamethoxazole
Retinoic Acid
Diazepam
Clonazepam
Temazepam
Ceftriaxone
Ceftriaxone
Midazolam
Midazolam
Interferon
Retinoic Acid
Ampicillin
Amoxicillin
Aspirin
Penicillin V
Tetracycline
Guaifenesin
Ceftriaxone
Ceftriaxone
Tetracycline
Warfarin
Interferon
Hydrochlorothiazide
Ibuprofen
Ethinyl Estradiol
Theophylline
Doxycycline
Ampicillin
Furosemide
Digoxin
Piroxicam
Piroxicam
Doxycycline
Erythromycin
Promethazine
Promethazine
Cyclobenzaprine
Ketoprofen

NAME

RS-3540
 RS-3650
 RS37619
 Ru-24756
 Rufen
 Rusyde
 Ru-Tuss
 Ru-Tuss
 Ru-Tuss Tablets
 Ru-Tuss Tablets
 Rynacrom
 S 1520
 S-6810 (Cys-Tyr-Cys-interferon-gamma)
 Sabidal
 Saizen
 Salacetin
 Salbulin
 Salbumol
 salbutamol
 Salbutard
 Salbutine
 Salbuvent
 Salcetogen
 Saletin
 salicylic acid acetate
 Salmetedur
 Salzone
 Samecin
 Sanasthmax
 Sanasthmyl
 Sanclomycine
 Sandimmun(e)
 Sandoptal
 Sanoma
 Saroten
 Sarotex
 Sartosona
 Sasulen
 Sawacillin
 Saxizon (hydrocortisone 21-sodium succinate)
 SC 11,800
 Sch 1000
 Sch 1000-Br-monohydrate
 Sch 4831
 Sch 11460 (betamethasone 17,21-dipropionate)
 Sch-13521
 Sch 13949W sulfate
 Sch 18020W
 Sch-29851
 SCH-30500 (Alfa-2b)
 Sch-32088
 Scheroson F

MONOGRAPH

Naproxen
 Naproxen
 Ketorolac
 Cefotaxime
 Ibuprofen
 Furosemide
 Guaifenesin
 Phenylpropanolamine
 Atropine
 Scopolamine
 Cromolyn
 Indapamide
 Interferon
 Theophylline
 Somatropin
 Aspirin
 Albuterol
 Albuterol
 Albuterol
 Albuterol
 Albuterol
 Albuterol
 Aspirin
 Aspirin
 Aspirin
 Salmeterol
 Acetaminophen
 Doxycycline
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Tetracycline
 Cyclosporine
 Butalbital
 Carisoprodol
 Amitriptyline
 Amitriptyline
 Cephalexin
 Piroxicam
 Amoxicillin
 Hydrocortisone
 Ethynodiol Diacetate
 Ipratropium Bromide
 Ipratropium Bromide
 Betamethasone
 Betamethasone
 Flutamide
 Albuterol
 Beclomethasone Dipropionate
 Loratadine
 Interferon
 Mometasone Furoate
 Hydrocortisone

NAME

Scop
 scopine tropate
 Scopoderm TTS
 scopolamine
l-scopolamine
 Scopos
 Scorprin
 SDandolanid
 SDM No. 17
 SDM No. 27
 SDM No. 37
 SE 1520
 Sebatrol
 Seclodin
 Sectorgel
 Securit
 Securon
 Sedapam
 Sedatival
 Sedazin
 Sedral
 Seduxen
 Seldane
 Selectin
 Seles Beta
 Selipran
 Selobloc
 Seloken
 Selopral
 Selo-Zok
 Semelente Iletin
 Semi-Daonil
 Semi-Euglucon
 Semi-Gliben-Puren N
 Semilente
 Sencephalin
 Sensaval
 Sensival
 Sepamit
 Septicide
 Septocillin
 Septra
 Septra
 Septrin
 Septrin
 D-Ser(Bu^t)⁶Azgly¹⁰-gonadorelin
 D-Ser(Bu^t)⁶Azgly¹⁰-luliberin
 Serevent
 Serono-Bagren
 Seroten
 sertraline
 Servisone

MONOGRAPH

Scopolamine
 Scopolamine
 Scopolamine
 Scopolamine
 Scopolamine
 Scopolamine
 Trimethoprim
 Digoxin
 Nitroglycerin
 Nitroglycerin
 Nitroglycerin
 Indapamide
 Flutamide
 Ibuprofen
 Ketoprofen
 Lorazepam
 Verapamil
 Diazepam
 Lorazepam
 Lorazepam
 Cefadroxil
 Diazepam
 Terfenadine
 Pravastatin
 Atenolol
 Pravastatin
 Atenolol
 Metoprolol
 Metoprolol
 Metoprolol
 Insulin
 Glyburide
 Glyburide
 Glyburide
 Insulin
 Cephalexin
 Nortriptyline
 Nortriptyline
 Nifedipine
 Ciprofloxacin
 Penicillin V
 Trimethoprim
 Sulfamethoxazole
 Sulfamethoxazole
 Trimethoprim
 Goserelin
 Goserelin
 Salmeterol
 Bromocriptine
 Amitriptyline
 Sertraline
 Prednisone

NAME

Servispor
 Setonil
 SF 86-327
 Sigacyclat
 Sigadoxin
 Sigamopen
 Sigapedil (erythromycin ethylsuccinate)
 Sigaprim
 Sigaprim
 Sigmacort (hydrocortisone 21-acetate)
 Signopam
 Silamox
 Simovil
 Simoxil
 Sinarest
 Sinarest
 Sinartról (piroxicam cinnamate)
 Sine-Off
 Sine-Off Sinus Medicine
 Sine-Off Sinus Medicine
 Sinomin
 Sintolexyr
 Sintotrat (hydrocortisone 21-acetate)
 Sinubid
 Sinubid
 Sinvacor
 Sirotol
 Sirtal
 Sivastin
 Sivlor
 SK 65
 SK-65 Apap
 SK-65 Compound
 SK-65 Compound
 SK-Erythromycin (erythromycin stearate)
 SKF 8542
 SKF 92334
 SK-Penicillin VK
 SL 80.0750
 SL 80.0750-23N
 Slo-Bid
 Slo-Phyllin
 Slo-Phyllin
 Slo-Phyllin GG
 SN 307
 S.N.G.
 Sobelin
 Sodelut G
 sodium ampicillin
 sodium [o-[(2,6-dichloropenyl)amino]
 phenyl]acetate

MONOGRAPH

Cephalixin
 Diazepam
 Terbinafine
 Doxycycline
 Doxycycline
 Amoxicillin
 Erythromycin
 Sulfamethoxazole
 Trimethoprim
 Hydrocortisone
 Temazepam
 Amoxicillin
 Simvastatin
 Amoxicillin
 Acetaminophen
 Phenylpropanolamine
 Piroxicam
 Acetaminophen
 Aspirin
 Phenylpropanolamine
 Sulfamethoxazole
 Cephalixin
 Hydrocortisone
 Acetaminophen
 Phenylpropanolamine
 Simvastatin
 Guaifenesin
 Carbamazepine
 Simvastatin
 Lovastatin
 Propoxyphene
 Propoxyphene
 Aspirin
 Propoxyphene
 Erythromycin
 Triamterene
 Cimetidine
 Penicillin V
 Zolpidem
 Zolpidem
 Theophylline
 Guaifenesin
 Theophylline
 Theophylline
 Ondansetron
 Nitroglycerin
 Clindamycin
 Medroxyprogesterone Acetate
 Ampicillin
 Diclofenac

NAME	MONOGRAPH
sodium (+)-(3 <i>R</i> ,5 <i>R</i>)-3,5-dihydroxy-7- [(1 <i>S</i> ,2 <i>S</i> ,6 <i>S</i> ,8 <i>S</i> ,8 <i>aR</i>)-6-hydroxy-2-methyl-8-[(<i>S</i>)- 2-methylbutyryloxy]-1,2,6,7,8,8 <i>a</i> -hexahydro-1- naphthyl]heptanoate	Pravastatin
sodium (±)-(3 <i>R</i> *,5 <i>S</i> *,6 <i>E</i>)-7-[3-(<i>p</i> -fluorophenyl)-1- isopropylindol-2-yl]-3,5-dihydroxy-6- heptenoate	Fluvastatin
sodium levothyroxine	Levothyroxine
Soframycin	Neomycin
Sofro	Cromolyn
Solantoin	Phenytoin
Solantyl	Phenytoin
Solestro (estradiol 3-benzoate)	Estradiol
Solis	Diazepam
Solocalm	Piroxicam
Solodelf (triamcinolone acetonide)	Triamcinolone
Solosin	Theophylline
Solprin	Aspirin
Solprofen	Ibuprofen
Solpyron	Aspirin
Solu-Cortef (hydrocortisone 21-sodium succinate)	Hydrocortisone
SoluGlyc (hydrocortisone 21-sodium succinate)	Hydrocortisone
Solu-Medrol (methylprednisolone 21-succinate)	Methylprednisolone
Solutadarol (triamcinolone acetonide 21- hemisuccinate)	Triamcinolone
Soma	Carisoprodol
Soma Compound	Aspirin
Soma Compound	Codeine
Somacton	Somatropin
Somadril	Carisoprodol
Somagerol	Lorazepam
Somalgit	Carisoprodol
somatropic hormone	Somatropin
somatropin	Somatropin
Somophyllin-T	Theophylline
Sone	Prednisone
Sostril	Ranitidine
SPA-S-510	Piroxicam
Spanor	Doxycycline
Spasmolytin	Adiphenine
Spectrazole	Cefuroxime
Spectrocin	Neomycin
Spectrum	Ceftazidime
SQ 9993 (estradiol 17-undecanoate)	Estradiol
SQ 14225	Captopril
SQ 16,150 (estradiol enanthate)	Estradiol
SQ-31000	Pravastatin
Stabicilline	Penicillin V
Stabillin V-K	Penicillin V
Stabisol	Methylprednisolone
Stacillin	Clavulanic Acid

NAME

Starcef
 Staticin
 Statrol
 Statrol
 Stazepine
 Steclin
 Stédiril
 Stédiril
 Stellamicina (erythromycin estolate)
 Stesolid
 Stesolin
 STH
 Stiemycin
 Stiliclina
 Stillacor
 Stilnoct
 Stilnox
 St.Joseph Aspirin for Adults
 St.Joseph's Cold Tablets
 Subamycin
 Succosa
 Sucralfin
 Sucrate
 sucrose octakis(hydrogen sulfate) aluminum
 complex
 Sudafed
 Sudafed Sinus
 Sugast
 sulbactam
 Sulcrate
 sulfamethoxazole
 sulfamethylisoxazole
N-sulfamoyl-3-[(2-guanidinothiazol-4-
 yl)methylthio]propionamide
N-(3-sulfamyl-4-chlorobenzamido)-2-
 methylindoline
 3-sulfanilamido-5-methylisoxazole
 Sulfatrim
 Sulfatrim
 sulfisomezole
 Sulfotrim
 Sulfotrim
 Sulfotrimin
 Sulfotrimin
 Sulperazone
 Sulprim
 Sulprim
 Sultanol
 Sumamed
 Sumapen VK
 sumatriptan
 Sumetrolim

MONOGRAPH

Ceftazidime
 Erythromycin
 Neomycin
 Polymyxin
 Carbamazepine
 Tetracycline
 Ethinyl Estradiol
 Norgestrel
 Erythromycin
 Diazepam
 Diazepam
 Somatropin
 Erythromycin
 Tetracycline
 Digoxin
 Zolpidem
 Zolpidem
 Aspirin
 Acetaminophen
 Tetracycline
 Sucralfate
 Sucralfate
 Sucralfate
 Sucralfate
 Guaifenesin
 Acetaminophen
 Sucralfate
 Sulbactam
 Sucralfate
 Sulfamethoxazole
 Sulfamethoxazole
 Famotidine
 Indapamide
 Sulfamethoxazole
 Trimethoprim
 Sulfamethoxazole
 Sulfamethoxazole
 Sulfamethoxazole
 Trimethoprim
 Sulfamethoxazole
 Trimethoprim
 Trimethoprim
 Sulbactam
 Sulfamethoxazole
 Trimethoprim
 Albuterol
 Azithromycin
 Penicillin V
 Sumatriptan
 Trimethoprim

NAME

Sumetrolim
 Sumiferon (Alfa-n1)
 Sumox
 Sumycin
 Supac
 Supac
 Suprax
 Supracombin
 Supracombin
 Supracyclin
 Suprametil
 Supramycin
 Suprax
 Supressin
 Suprim
 Suprim
 Supristol
 Suractin
 Susadrin
 Suscard
 Suspen
 Suspren
 Sustac
 Sustaire
 Sustamycin
 Sustonit
 Suxizon 300 (hydrocortisone 21-sodium succinate)
 Sylvemid
 Synacort
 Synalgos
 Syncl
 Synflex
 Synkonin
 Synpenin
 Synphase
 Synphase
 Synthroid
 Synthroid Sodium
 Synutrim
 synvinolin
 Syraprim
 System
 Tabalgin
 Tabalon
 TAC-3 (triamcinolone acetonide)
 TAC-40 (triamcinolone acetonide)
 TAC-D (triamcinolone diacetate)
 Tacosal
 Tacumil
 Tacumil
 Tafil

MONOGRAPH

Sulfamethoxazole
 Interferon
 Amoxicillin
 Tetracycline
 Aspirin
 Acetaminophen
 Cefixime
 Sulfamethoxazole
 Trimethoprim
 Doxycycline
 Methylprednisolone
 Tetracycline
 Cefixime
 Doxazosin
 Trimethoprim
 Sulfamethoxazole
 Trimethoprim
 Ampicillin
 Nitroglycerin
 Nitroglycerin
 Penicillin V
 Ibuprofen
 Nitroglycerin
 Theophylline
 Tetracycline
 Nitroglycerin
 Hydrocortisone

 Amitriptyline
 Hydrocortisone
 Aspirin
 Cephalixin
 Naproxen
 Hydrocodone
 Ampicillin
 Ethinyl Estradiol
 Norethindrone
 Levothyroxine
 Levothyroxine
 Trimethoprim
 Simvastatin
 Trimethoprim
 Estradiol
 Acetaminophen
 Ibuprofen
 Triamcinolone
 Triamcinolone
 Triamcinolone
 Phenytoin
 Trimethoprim
 Sulfamethoxazole
 Alprazolam

NAME

Tagamet
 Taicelexin
 Talotren
 Talsutin
 Tametin
 Tanamicin
 Tandix
 TAP-144
 Tapar
 Tarasyn
 Tarivid
 TATBA (triamcinolone hexacetonide)
 Taural
 Tavor
 taxol
 taxol A
 Tazicef
 Tazidime
 TBI (triamcinolone benetonide)
 TE-031
 Tecacin
 Tecodin
 Tedolan
 Tedral
 Tedral Expectorant
 Teen Midol
 Tefilin
 Tegretal
 Tegretol
 Tekodin
 Teldane
 Teldanex
 Teleprim
 Teleprim
 Telesmin
 Teline
 Telotrex
 Temesta
 Temlo
 Temporinolo
 Tempra
 Temserin
 Tenacid
 Tennuss
 Teno-basan
 Tenoblock
 Tenopt
 Tenoretic
 Tenormin
 Tensobon
 Tensoprel
 Tensopril

MONOGRAPH

Cimetidine
 Cephalexin
 Theophylline
 Tetracycline
 Cimetidine
 Doxycycline
 Indapamide
 Leuprolide
 Acetaminophen
 Ketorolac
 Ofloxacin
 Triamcinolone
 Ranitidine
 Lorazepam
 Paclitaxel
 Paclitaxel
 Ceftazidime
 Ceftazidime
 Triamcinolone
 Clarithromycin
 Doxycycline
 Oxycodone
 Etodolac
 Theophylline
 Guaifenesin
 Acetaminophen
 Tetracycline
 Carbamazepine
 Carbamazepine
 Oxycodone
 Terfenadine
 Terfenadine
 Trimethoprim
 Sulfamethoxazole
 Carbamazepine
 Tetracycline
 Tetracycline
 Lorazepam
 Acetaminophen
 Phenylpropanolamine
 Acetaminophen
 Timolol
 Imipenem
 Guaifenesin
 Atenolol
 Atenolol
 Timolol
 Atenolol
 Atenolol
 Atenolol
 Captopril
 Captopril
 Lisinopril

NAME

Theocontin
 Theo-Dur
 Theograd
 Theolair
 Theolair Plus
 Theolair Plus
 Theolan
 Theolix
 Theon
 Theo-Organidin
 Theophyl
 Theoplus
 Theopropanol
 Theo-Sav
 Theosol
 Theostat
 Theovent
 TheraFlu
 Thiaretic
 Thiergan
 Thilophenyt
 Thiocuran
 Thiocuran
 Thiuretic
 Thyroxevan
 thyroxine
 L-thyroxine sodium salt
 Tibatin
 Tibicorten (triamcinolone benetonide)
 Tibricol
 Tiempe
 Tienam
 Tildiem
 Timacar
 Timacor
 Timolate
 Timentin
 Timocort
 Timolide
 Timolide
 Timonil
 Timoptic
 Timoptol
 Tintorane
 Tiplagen
 tissue plasminogen activator
 TMS 480
 TMS 480
 Togiren (erythromycin estolate)
 Tokiocillin
 Tokiolexin
 Tokuderm (betamethasone 17-valerate)

MONOGRAPH

Theophylline
 Theophylline
 Theophylline
 Theophylline
 Guaifenesin
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Theophylline
 Acetaminophen
 Hydrochlorothiazide
 Promethazine
 Phenytoin
 Sulfamethoxazole
 Trimethoprim
 Hydrochlorothiazide
 Levothyroxine
 Levothyroxine
 Levothyroxine
 Clotrimazole
 Triamcinolone
 Nifedipine
 Trimethoprim
 Imipenem
 Diltiazem
 Timolol
 Timolol
 Timolol
 Timolol
 Clavulanic Acid
 Hydrocortisone
 Hydrochlorothiazide
 Timolol
 Carbamazepine
 Timolol
 Timolol
 Warfarin
 Alteplase
 Alteplase
 Trimethoprim
 Sulfamethoxazole
 Erythromycin
 Ampicillin
 Cephalexin
 Betamethasone

NAME

Tolycar
 Topagen (betamethasone 17-valerate)
 Topicycline
 Toprec
 Toprek
 Toprol-XL
 Toradol
 Toratex
 Torental
 Torlamicina
 Totacillin
 Totalciclina
 Totapen
 Totomycin
 Tova
 TPA
 T-Phyl
 Tracilon (triamcinolone diacetate)
 Tracix
 Trafarbiot
 Tralgon
 Tramacin (triamcinolone acetonide)
 tramadol
 Tramal
 Tranimul
 Trankimazin
 Tranquase
 Tranquinal
 Tranquo-Puren
 Tranquo-Tablinen
 Transcop
 Transderm-Nitro
 Transderm-Nitro TTS
 Transderm-Scop
 Transderm-V
 Transit
 Trantoin
 Trasentine hydrochloride
 Tratul
 Travin
 Trendar
 Trental
 Tresaderm
 tretinoin
 triaconazole
 Triam (triamcinolone acetonide)
 Triamcet (triamcinolone acetonide)
 Triamcin (triamcinolone diacetate)
 Triaminic
 Triaminic
 2,4,7-triamino-6-phenylpteridine
 Triamolone 40 (triamcinolone diacetate)

MONOGRAPH

Cefotaxime
 Betamethasone
 Tetracycline
 Ketoprofen
 Ketoprofen
 Metoprolol
 Ketorolac
 Ketorolac
 Pentoxifylline
 Erythromycin
 Ampicillin
 Ampicillin
 Ampicillin
 Tetracycline
 Ethinyl Estradiol
 Alteplase
 Theophylline
 Triamcinolone
 Imipenem
 Ampicillin
 Acetaminophen
 Triamcinolone
 Tramadol
 Tramadol
 Diazepam
 Alprazolam
 Diazepam
 Alprazolam
 Diazepam
 Diazepam
 Diazepam
 Scopolamine
 Nitroglycerin
 Nitroglycerin
 Scopolamine
 Scopolamine
 Furosemide
 Nitrofurantoin
 Adiphenine
 Cimetidine
 Buspirone
 Ibuprofen
 Pentoxifylline
 Neomycin
 Retinoic Acid
 Terconazole
 Triamcinolone
 Triamcinolone
 Triamcinolone
 Guaifenesin
 Phenylpropanolamine
 Triamterene
 Triamcinolone

NAME	MONOGRAPH
Triamnone 40 (triamcinolone acetonide)	Triamcinolone
Triatec	Ramipril
Triavil	Amitriptyline
Tribrissen	Trimethoprim
Tricinolon (triamcinolone acetonide)	Triamcinolone
Tricortale	Triamcinolone
Tridil	Nitroglycerin
Tri-Ervonum	Ethinyl Estradiol
Triflucan	Fluconazole
α,α,α -trifluoro-2-methyl-4'-nitro- <i>m</i> -propionotoluidine	Flutamide
Trigger	Ranitidine
Triglobe	Trimethoprim
Trigonyl	Sulfamethoxazole
Trigonyl	Trimethoprim
11,17,21-trihydroxy-6-methyl-1,4-pregnadiene-3,20-dione	Methylprednisolone
11,17,21-trihydroxypregn-4-ene-3,20-dione	Hydrocortisone
Tri-Levlen	Norgestrel
Tri-Levlen	Ethinyl Estradiol
Triludan	Terfenadine
Trimanyl	Trimethoprim
Trimesulf	Sulfamethoxazole
Trimesulf	Trimethoprim
5-[(3,4,5-trimethoxyphenyl)methyl]-2,4-pyrimidinediamine	Trimethoprim
<i>N,N</i> ,6-trimethyl-2-(4-methylphenyl)imidazo[1,2- <i>a</i>]pyridine-3-acetamide	Zolpidem
<i>N,N</i> , α -trimethyl-10 <i>H</i> -phenothiazine-10-ethanamine	Promethazine
<i>N,N</i> ,6-trimethyl-2- <i>p</i> -tolylimidazo[1,2- <i>a</i>]pyridine-3-acetamide	Zolpidem
Trimforte	Trimethoprim
Trimforte	Sulfamethoxazole
Tri-Minulet	Ethinyl Estradiol
Trimogal	Trimethoprim
Trimopan	Trimethoprim
Trimox	Amoxicillin
Trimpex	Trimethoprim
Trimysten	Clotrimazole
Trinalgon	Nitroglycerin
Trind	Phenylpropanolamine
trinitrin	Nitroglycerin
trinitroglycerol	Nitroglycerin
Trinitrosan	Nitroglycerin
Trinordiol	Ethinyl Estradiol
Trinordiol	Norgestrel
Tri-Norinyl	Ethinyl Estradiol
Tri-Norinyl	Norethindrone
Trinovum	Ethinyl Estradiol
Trinovum	Norethindrone
Triphacyclin	Tetracycline

NAME

Triphasil
 Triphasil
 Triptizol
 Tritace
 Triteren
 Trofurit
 1 α H,5 α H-tropan-3 α -ol (\pm)-tropate
 tropic acid ester with tropine
 tropic acid ester with scopine
 tropine tropate
 troyl tropate
 trozocina
 Trymex (triamcinolone acetone)
 Tryptanol
 Tryptizol
 TS 408 (hydrocortisone butepate)
 tsiklomitsin
 T-Stat
 Tsudohmin
 Tuly
 Turixin
 Tussar-2
 Tussar-2
 Tussar-SF
 Tussar-SF
 Tussi-Organidin
 Tuss-Ornade
 Tuttomycin
 Tylenol
 Tylenol
 Tylox
 Tylox
 U-18,573
 U-18,573G
 U-26,225A
 U-26452
 U-31889
 U-75630 (ibuprofen piconol)
 UK-33274
 UK-33274-27
 UK-48340
 UK-48340-11
 UK-48340-26
 UK 49858
 Ukapen
 Ulcar
 Ulcedin
 Ulcedine
 Ulceprax
 Ulcerban
 Ulcerfen
 Ulcerlmin

MONOGRAPH

Ethinyl Estradiol
 Norgestrel
 Amitriptyline
 Ramipril
 Triamterene
 Furosemide
 Atropine
 Atropine
 Scopolamine
 Atropine
 Atropine
 Azithromycin
 Triamcinolone
 Amitriptyline
 Amitriptyline
 Hydrocortisone
 Tetracycline
 Erythromycin
 Diclofenac
 Guaifenesin
 Mupirocin
 Codeine
 Guaifenesin
 Guaifenesin
 Codeine
 Codeine
 Phenylpropanolamine
 Neomycin
 Acetaminophen
 Codeine
 Acetaminophen
 Oxycodone
 Ibuprofen
 Ibuprofen
 Tramadol
 Glyburide
 Alprazolam
 Ibuprofen
 Doxazosin
 Doxazosin
 Amlodipine
 Amlodipine
 Amlodipine
 Fluconazole
 Ampicillin
 Sucralfate
 Cimetidine
 Cimetidine
 Famotidine
 Sucralfate
 Cimetidine
 Sucralfate

NAME

Ulcex
 Ulcimet
 Ulcofalk
 Ulcogant
 Ulcomedina
 Ulcomet
 Ulfamid
 Ulfinol
 Ulhys
 Ultidine
 Ultrabion
 Ultracef
 Ultracorten
 Ultracortene
 Ultradol
 Ultralente
 Ultralente Iletin
 Ultram
 Ultroxim
 Umatrope
 Umipres
 Unacid
 Unacil
 Unacim
 Unasyn
 Unasyn(a)
 Unibloc
 Unicam
 Unicin
 Uni-Dur
 Unifyl
 Uniloc
 Unimycin
 Uniphyl
 Uniphyllin
 Unipril
 Unisedil
 Unistradiol (estradiol 3-benzoate)
 Univer
 Unixime
 Upcyclin
 Urantoin
 Urbason
 Urbason-Solubile (methylprednisolone 21-succinate)
 Urem
 Uretrim
 Urex
 Urigon
 Urisedamine
 Urizept
 Urocaudal

MONOGRAPH

Ranitidine
 Cimetidine
 Cimetidine
 Sucralfate
 Cimetidine
 Cimetidine
 Famotidine
 Famotidine
 Cimetidine
 Ranitidine
 Ampicillin
 Cefadroxil
 Prednisone
 Prednisone
 Etodolac
 Insulin
 Insulin
 Tramadol
 Cefuroxime
 Somatropin
 Hydrochlorothiazide
 Sulbactam
 Doxycycline
 Sulbactam
 Ampicillin
 Sulbactam
 Atenolol
 Piroxicam
 Tetracycline
 Theophylline
 Theophylline
 Atenolol
 Tetracycline
 Theophylline
 Theophylline
 Ramipril
 Diazepam
 Estradiol
 Verapamil
 Cefixime
 Tetracycline
 Nitrofurantoin
 Methylprednisolone
 Methylprednisolone

 Ibuprofen
 Trimethoprim
 Furosemide
 Diclofenac
 Atropine
 Nitrofurantoin
 Triamterene

NAME

Uro-Clamoxyl
 Urodiazin
 Urodie
 Urodil
 Urodin
 Urolong
 Uroplus
 Uroplus
 Urosemide
 Uro-Septra
 Uro-Septra
 Uro-Tablinen
 Uticillin VK
 Uticort (betamethasone 17-benzoate)
 Utimox
 Utovlan
 V-Cil
 V-Cil-K
 V-Cillin
 V-Cillin K
 Vagifen
 Valadol
 Valaxona
 Valcote
 valergen (estradiol 17-valerate)
 Valetan
 Valiquid
 Valisone (betamethasone 17-valerate)
 Valium
 VallerGINE
 Valmagen
 Valrelease
 Vancenase
 Vanceril
 Vancocin
 Vancoled
 vancomycin
 Vancor
 Vanoxin
 Vanquish
 Vanquish
 Varnoline
 Varnoline
 Vaseretic
 Vaseretic
 Vasocard
 Vasoglyn
 Vasolan
 Vasomet
 Vasotec
 Vasten
 vazofirin

MONOGRAPH

Amoxicillin
 Hydrochlorothiazide
 Terazosin
 Nitrofurantoin
 Nitrofurantoin
 Nitrofurantoin
 Trimethoprim
 Sulfamethoxazole
 Furosemide
 Trimethoprim
 Sulfamethoxazole
 Nitrofurantoin
 Penicillin V
 Betamethasone
 Amoxicillin
 Norethindrone
 Penicillin V
 Penicillin V
 Penicillin V
 Penicillin V
 Estradiol
 Acetaminophen
 Diazepam
 Valproic Acid
 Estradiol
 Diclofenac
 Diazepam
 Diazepam
 Betamethasone
 Diazepam
 Promethazine
 Cimetidine
 Diazepam
 Beclomethasone Dipropionate
 Beclomethasone Dipropionate
 Vancomycin
 Vancomycin
 Vancomycin
 Vancomycin
 Digoxin
 Acetaminophen
 Aspirin
 Desogestrel
 Ethinyl Estradiol
 Enalapril
 Hydrochlorothiazide
 Terazosin
 Nitroglycerin
 Verapamil
 Terazosin
 Enalapril
 Pravastatin
 Pentoxifylline

NAME	MONOGRAPH
Vebecillin	Penicillin V
Veclam	Clarithromycin
Veetids	Penicillin V
Velmonit	Ciprofloxacin
Velopural (hydrocortisone 21-acetate)	Hydrocortisone
Velosulin	Insulin
Venetlin	Albuterol
venlafaxine	Venlafaxine
venlafaxine	Venlafaxine
Venopex	Cimetidine
Venter	Sucralfate
Ventodisks	Albuterol
Ventolin	Albuterol
Volma	Albuterol
Vepen	Penicillin V
Vepesid	Etoposide
Veracim	Verapamil
Veradol	Naproxen
Veramex	Verapamil
Veramix	Medroxyprogesterone Acetate
Veraptin	Verapamil
Verelan	Verapamil
Verexamil	Verapamil
Veroxil	Indapamide
Versacort (hydrocortisone bendazac)	Hydrocortisone
Versed	Midazolam
Vesdil	Ramipril
Vesnaroid (tretinoin)	Retinoic Acid
Vesuprim	Trimethoprim
Vetacortyl (methylprednisolone 21-acetate)	Methylprednisolone
Vetalog (triamcinolone acetate)	Triamcinolone
Vetidrex	Hydrochlorothiazide
Vetquamacin-324	Tetracycline
Viarex	Beclomethasone Dipropionate
Viarox	Beclomethasone Dipropionate
Vibradox	Doxycycline
Vibramycin	Doxycycline
Vibramycin hyclate	Doxycycline
Vibra-Tabs	Doxycycline
Vibraveineuse	Doxycycline
Vibravenös	Doxycycline
Vicard	Terazosin
Viccillin	Ampicillin
Vicodin	Acetaminophen
Vicodin	Hydrocodone
Vidopen	Ampicillin
Vioform-Hydrocortisone	Hydrocortisone
Vipral	Acyclovir
Viraferon (Alfa-2b)	Interferon
Virorax	Acyclovir
Visiren	Ofloxacin

NAME	MONOGRAPH
Vista-Methasone (betamethasone 21-phosphate disodium salt)	Betamethasone
Visubeta	Betamethasone
vitamin A acid (tretinoin)	Retinoic Acid
Vival	Diazepam
Vivatec	Lisinopril
Vividrin	Cromolyn
Vividyl	Nortriptyline
Vivol	Diazepam
Vivox	Doxycycline
Voldal	Diclofenac
Volidan	Ethinyl Estradiol
Volmax	Albuterol
Volon	Triamcinolone
Volon A (triamcinolone acetonide)	Triamcinolone
Volonimat (triamcinolone acetonide)	Triamcinolone
Voltaren	Diclofenac
Voltarol	Diclofenac
Vonamycin Powder V	Neomycin
VoSol HC	Hydrocortisone
Voveran	Diclofenac
VP-16-213	Etoposide
Vytone	Hydrocortisone
W 5975 (betamethasone 17-benzoate)	Betamethasone
Waran	Warfarin
WARF compound 42	Warfarin
warfarin	Warfarin
warfarin-deanol	Warfarin
Warfilone	Warfarin
Waruzol	Warfarin
Welfurin	Astemizole
Wellcome 248U	Nitrofurantoin
Wellcoprim	Acyclovir
Wellferon (Alfa-n1)	Trimethoprim
Wemid (erythromycin stearate)	Interferon
Weradys	Erythromycin
Westcort Cream (hydrocortisone 17-valerate)	Ethinyl Estradiol
Westcort (hydrocortisone 17-valerate)	Hydrocortisone
Widecillin	Hydrocortisone
Wy 3467	Amoxicillin
Wy-3707	Diazepam
Wy 3917	Norgestrel
Wy-4036	Temazepam
Wy 21743	Lorazepam
WY-45030	Oxaprozin
WY-45651	Venlafaxine
WY-45655	Venlafaxine
Wyamycin E (erythromycin ethylsuccinate)	Venlafaxine
Wygesic	Erythromycin
Wymox	Propoxyphene
Wyovin Hydrochloride	Amoxicillin
Wypax	Dicyclomine
	Lorazepam

NAME	MONOGRAPH
Xahl	Cephalexin
Xanax	Alprazolam
Xanef	Enalapril
Xanor	Alprazolam
Xanthium	Theophylline
Xaxa	Aspirin
Xenar	Naproxen
Xenid	Diclofenac
Ximos (cefuroxime axetil)	Cefuroxime
Xiphen	Pentoxifylline
XL-90	Guaifenesin
XU 62-320	Fluvastatin
Xynertec	Enalapril
XZ-450	Azithromycin
Yermonil	Ethinyl Estradiol
YM-11170	Famotidine
YM-14090 (Alfa-2b)	Interferon
Zadorin	Doxycycline
Zamocillin	Amoxicillin
Zanizal	Nizatidine
Zantac	Ranitidine
Zantic	Ranitidine
Zantidon	Ranitidine
ZE-101	Nizatidine
Zeclar	Clarithromycin
Zedolac	Etodolac
Zelis (piroxicam cinnamate)	Piroxicam
Zen (piroxicam cinnamate)	Piroxicam
Zenoxone	Hydrocortisone
Zentropil	Phenytoin
Zenusin	Nifedipine
Zestril	Lisinopril
Zienam	Cilastatin
Zienam	Imipenem
Zinacef	Cefuroxime
Zinat (cefuroxime axetil)	Cefuroxime
Zinnat (cefuroxime axetil)	Cefuroxime
Zithromax	Azithromycin
Zitromax	Azithromycin
ZL-101	Nizatidine
Zocor	Simvastatin
Zocord	Simvastatin
Zofran	Ondansetron
Zoladex	Goserelin
Zoloft	Sertraline
zolpidem	Zolpidem
Zoofurin	Nitrofurantoin
Zophren	Ondansetron
Zorprin	Aspirin
Zovirax	Acyclovir

NAME

Zumenon
Zunden
Zydol
Zydone
Zydone

MONOGRAPH

Estradiol
Piroxicam
Tramadol
Acetaminophen
Hydrocodone

MOLECULAR FORMULA INDEX

In general, molecular formulae are given for the simplest form of the compound. Thus, although a particular drug might be used as the hydrochloride, the molecular formula will refer to the free base.

MOLECULAR FORMULA	COMPOUND
$C_3H_5N_3O_9$	Nitroglycerin
$C_6H_{12}N_2O_4Pt$	Carboplatin
$C_7H_8ClN_3O_4S_2$	Hydrochlorothiazide
$C_7H_8N_4O_2$	Theophylline
$C_8H_{11}NO_5S$	Sulbactam
$C_8H_{11}N_5O_3$	Acyclovir
$C_8H_{15}N_7O_2S_3$	Famotidine
$C_8H_{16}O_2$	Valproic acid
$C_8H_6N_4O_5$	Nitrofurantoin
$C_8H_9NO_2$	Acetaminophen
$C_8H_9NO_5$	Clavulanic acid
$C_9H_7N_7O_2S$	Azathioprine
$C_9H_9O_4$	Aspirin
$C_9H_{14}ClNO$	Phenylpropanolamine
$C_9H_{15}NO_3S$	Captopril
$C_{10}H_{11}N_3O_3S$	Sulfamethoxazole
$C_{10}H_{13}N_5O_4$	Zidovudine
$C_{10}H_{14}O_4$	Guaifenesin
$C_{10}H_{16}N_6S$	Cimetidine
$C_{11}H_{11}F_3N_2O_3$	Flutamide
$C_{11}H_{16}N_2O_3$	Butalbital
$C_{12}H_{11}ClN_2O_5S$	Furosemide
$C_{12}H_{11}N_7$	Triamterene
$C_{12}H_{17}N_3O_4S$	Imipenem
$C_{12}H_{18}O$	Propofol
$C_{12}H_{21}N_5O_2S_2$	Nizatidine
$C_{12}H_{24}N_2O_4$	Carisoprodol
$C_{12}H_{54}Al_{16}O_{75}S_8$	Sucalfate
$C_{13}H_{12}F_2N_6O$	Fluconazole
$C_{13}H_{16}N_4O_3$	Pentoxifylline
$C_{13}H_{18}O_2$	Ibuprofen
$C_{13}H_{21}NO_3$	Albuterol

MOLECULAR FORMULA	COMPOUND
$C_{13}H_{22}N_4O_3S$	Ranitidine
$C_{13}H_{24}N_4O_3S$	Timolol
$C_{14}H_{11}Cl_2NO_2$	Diclofenac
$C_{14}H_{14}O_3$	Naproxen
$C_{14}H_{18}N_4O_3$	Trimethoprim
$C_{14}H_{19}NO_2$	Methylphenidate
$C_{14}H_{21}N_3O_2S$	Sumatriptan
$C_{14}H_{22}N_2O_3$	Atenolol
$C_{15}H_{10}ClN_3O_3$	Clonazepam
$C_{15}H_{10}Cl_2N_2O_2$	Lorazepam
$C_{15}H_{11}I_4NO_4$	Levothyroxine
$C_{15}H_{12}N_2O$	Carbamazepine
$C_{15}H_{12}N_2O_2$	Phenytoin
$C_{15}H_{13}NO_3$	Ketorolac
$C_{15}H_{13}N_3O_4S$	Piroxicam
$C_{15}H_{14}ClN_3O_4S$	Cefaclor
$C_{15}H_{16}O_2$	Nabumetone
$C_{15}H_{22}O_3$	Gemfibrozil
$C_{15}H_{25}NO_3$	Metoprolol
$C_{16}H_{13}ClN_2O$	Diazepam
$C_{16}H_{13}ClN_2O_2$	Temazepam
$C_{16}H_{14}O_3$	Ketoprofen
$C_{16}H_{15}N_5O_7S_2$	Cefixime
$C_{16}H_{16}ClN_3O_3S$	Indapamide
$C_{16}H_{16}ClN_3O_4$	Loracarbef
$C_{16}H_{16}N_4O_8S$	Cefuroxime
$C_{16}H_{17}N_3O_4S$	Cephalexin
$C_{16}H_{17}N_3O_5S$	Cefadroxil
$C_{16}H_{17}N_5O_7S_2$	Cefotaxime
$C_{16}H_{18}N_2O_5S$	Penicillin V
$C_{16}H_{19}N_3O_4S$	Ampicillin
$C_{16}H_{19}N_3O_5S$	Amoxicillin
$C_{16}H_{25}NO_2$	Tramadol
$C_{16}H_{26}N_2O_5S$	Cilastatin
$C_{17}H_{13}ClN_4$	Alprazolam
$C_{17}H_{17}Cl_2N$	Sertraline
$C_{17}H_{18}FN_3O_3$	Ciprofloxacin
$C_{17}H_{18}F_3NO$	Fluoxetine
$C_{17}H_{18}N_2O_6$	Nifedipine
$C_{17}H_{19}N_3O_3S$	Omeprazole
$C_{17}H_{20}N_2S$	Promethazine
$C_{17}H_{21}NO_3$	Etodolac
$C_{17}H_{21}NO_4$	Scopolamine
$C_{17}H_{23}NO_3$	Atropine
$C_{17}H_{27}NO_2$	Venlafaxine
$C_{18}H_{13}ClFN_3$	Midazolam
$C_{18}H_{15}NO_3$	Oxaprozin
$C_{18}H_{18}N_6O_7S_3$	Ceftriaxone
$C_{18}H_{18}O_2$	Estrogens, conjugated (equilenin)
$C_{18}H_{19}ClN_4$	Clozapine
$C_{18}H_{19}N_3O$	Ondansetron
$C_{18}H_{19}N_3O_5S$	Cefprozil

MOLECULAR FORMULA

$C_{18}H_{20}FN_3O_4$
 $C_{18}H_{20}O_2$
 $C_{18}H_{21}NO_3$
 $C_{18}H_{21}NO_3$
 $C_{18}H_{21}NO_4$
 $C_{18}H_{22}O_2$
 $C_{18}H_{22}O_2$
 $C_{18}H_{24}O_2$
 $C_{18}H_{33}ClN_2O_5S$
 $C_{19}H_{16}O_4$
 $C_{19}H_{21}N$
 $C_{19}H_{21}N_3O$
 $C_{19}H_{25}N_5O_4$
 $C_{19}H_{35}NO_2$
 $C_{20}H_{21}N$
 $C_{20}H_{23}N$
 $C_{20}H_{24}O_2$
 $C_{20}H_{25}ClN_2O_5$
 $C_{20}H_{25}NO_2$
 $C_{20}H_{26}O_2$
 $C_{20}H_{28}N_2O_5$
 $C_{20}H_{28}O_2$
 $C_{20}H_{30}BrNO_3$
 $C_{21}H_{25}N$
 $C_{21}H_{26}O_5$
 $C_{21}H_{27}FO_6$
 $C_{21}H_{27}N_5O_4S$
 $C_{21}H_{28}O_2$
 $C_{21}H_{30}O_5$
 $C_{21}H_{31}N_3O_5$
 $C_{21}H_{31}N_5O_2$
 $C_{22}H_{17}ClN_2$
 $C_{22}H_{22}N_6O_7S_2$
 $C_{22}H_{23}ClN_2O_2$
 $C_{22}H_{24}N_2O_8$
 $C_{22}H_{24}N_2O_8$
 $C_{22}H_{26}N_2O_4S$
 $C_{22}H_{29}FO_5$
 $C_{22}H_{29}NO_2$
 $C_{22}H_{30}O$
 $C_{22}H_{30}O_5$
 $C_{23}H_{16}O_{11}$
 $C_{23}H_{25}N_5O_5$
 $C_{23}H_{28}ClN_3O_5S$
 $C_{23}H_{29}ClFN_3O_4$
 $C_{23}H_{32}N_2O_5$
 $C_{23}H_{36}O_7$
 $C_{23}H_{36}N_2O_2$
 $C_{23}H_{46}N_6O_{13}$
 $C_{24}H_{26}FNO_4$
 $C_{24}H_{28}N_2O_5$
 $C_{24}H_{32}O_4$

COMPOUND

Ofloxacin
 Estrogens, conjugated (equilin)
 Codeine
 Hydrocodone
 Oxycodone
 Estrogens, conjugated (estrone)
 Estrogens, conjugated (17 α -dihydroequilin)
 Estradiol; Estrogens, conjugated
 Clindamycin
 Warfarin
 Nortriptyline
 Zolpidem
 Terazosin
 Dicyclomine
 Cyclobenzaprine
 Amitriptyline
 Ethinyl estradiol
 Amlodipine
 Adiphenine
 Norethindrone
 Enalapril
 Retinoic acid
 Ipratropium bromide
 Terbinafine
 Prednisone
 Triamcinolone
 Glipizide
 Norgestrel
 Hydrocortisone
 Lisinopril
 Buspirone
 Clotrimazole
 Cefazidime
 Loratadine
 Tetracycline
 Doxycycline
 Diltiazem
 Betamethasone
 Propoxyphene
 Desogestrel
 Methylprednisolone
 Cromolyn
 Doxazosin
 Glyburide
 Cisapride
 Ramipril
 Pravastatin
 Finasteride
 Neomycin B
 Fluvastatin
 Benazepril
 Ethynodiol diacetate

MOLECULAR FORMULA	COMPOUND
$C_{24}H_{34}O_4$	Medroxyprogesterone acetate
$C_{24}H_{36}O_5$	Lovastatin
$C_{25}H_{30}N_2O_5$	Quinapril
$C_{25}H_{37}NO_4$	Salmeterol
$C_{25}H_{38}O_5$	Simvastatin
$C_{26}H_{28}Cl_2N_4O_4$	Ketoconazole
$C_{26}H_{31}Cl_2N_5O_3$	Terconazole
$C_{26}H_{44}O_9$	Mupirocin
$C_{27}H_{30}Cl_2O_6$	Mometasone furoate
$C_{27}H_{38}N_2O_4$	Verapamil
$C_{28}H_{31}FN_4O$	Astemizole
$C_{28}H_{37}ClO_7$	Beclomethasone dipropionate
$C_{29}H_{32}O_{13}$	Etoposide
$C_{32}H_{40}BrN_5O_5$	Bromocriptine
$C_{32}H_{41}NO_2$	Terfenadine
$C_{37}H_{67}NO_{13}$	Erythromycin
$C_{38}H_{69}NO_{13}$	Clarithromycin
$C_{38}H_{72}N_2O_{12}$	Azithromycin
$C_{41}H_{64}O_{14}$	Digoxin
$C_{47}H_{51}NO_{14}$	Paclitaxel
$C_{56}H_{98}N_{16}O_{13}$	Polymyxin B ₁
$C_{59}H_{84}N_{16}O_{12}$	Leuprolide
$C_{59}H_{84}N_{18}O_{14}$	Goserelin
$C_{62}H_{111}N_{11}O_{12}$	Cyclosporine
$C_{66}H_{75}Cl_2N_9O_{24}$	Vancomycin
$C_{258}H_{383}N_{65}O_{77}S_6$	Insulin (human)
$C_{809}H_{1301}N_{229}O_{240}S_5$	Epoetin (alfa)
$C_{860}H_{1353}N_{229}O_{255}S_9$	Interferon (alfa-2B)
$C_{990}H_{1528}N_{262}O_{300}S_7$	Somatropin
$C_{2736}H_{4174}N_{914}O_{824}S_{45}$	Alteplase

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50-03-3	Hydrocortisone acetate
50-23-7	Hydrocortisone
50-28-2	Estradiol; Estrogens conjugated
50-42-0	Adiphenine hydrochloride
50-48-6	Amitriptyline
50-50-0	Estradiol benzoate
50-78-2	Aspirin
51-34-3	Scopolamine
51-48-9	Levothyroxine
51-55-8	Atropine
51-98-9	Norethindrone acetate
52-28-8	Codeine phosphate
52-53-9	Verapamil
52-88-0	Atropine methylnitrate
53-03-2	Prednisone
53-16-7	Estrogens, conjugated (estrone)
53-36-1	Methylprednisolone acetate
54-31-9	Furosemide
54-87-5	Nitrofurantoin, sodium salt
55-03-8	Levothyroxine sodium
55-63-0	Nitroglycerin
57-41-0	Phenytoin
57-63-6	Ethinyl estradiol
57-91-0	Estradiol; Estrogens, conjugated
58-33-3	Promethazine hydrochloride
58-55-9	Theophylline
58-93-5	Hydrochlorothiazide
60-54-8	Tetracycline
60-87-7	Promethazine
64-75-5	Tetracycline hydrochloride
64-95-9	Adiphenine
67-20-9	Nitrofurantoin
67-78-7	Triamcinolone diacetate
67-92-5	Dicyclomine hydrochloride
68-22-4	Norethindrone
69-52-3	Ampicillin, sodium salt

CAS REGISTRY

NUMBER	COMPOUND
69-53-4	Ampicillin
71-58-9	Medroxyprogesterone acetate
72-69-5	Nortriptyline
76-25-5	Triamcinolone acetonide
76-42-6	Oxycodone
76-57-3	Codeine
77-19-0	Dicyclimine
77-26-9	Butalbital
78-44-4	Carisoprodol
81-81-2	Warfarin
83-43-2	Methylprednisolone
87-08-1	Penicillin V
93-14-1	Guaifenesin
99-66-1	Valproic acid
101-31-5	Atropine (hyoscyamine)
103-90-2	Acetaminophen
113-38-2	Estradiol dipropionate
113-45-1	Methylphenidate
114-07-8	Erythromycin
114-49-8	Scopolamine hydrobromide anhydrous
124-90-3	Oxycodone hydrochloride
124-94-7	Triamcinolone
125-04-2	Hydrocortisone 21-sodium succinate
125-25-7	Codeine hydrobromide
125-27-9	Codeine methyl bromide
125-29-1	Hydrocodone
129-06-6	Warfarin sodium
132-98-9	Penicillin V, potassium salt
134-36-1	Erythromycin propionate
143-71-5	Hydrocodone bitartrate
151-73-5	Betamethasone sodium phosphate
152-11-4	Verapamil hydrochloride
154-41-6	Phenylpropanolamine hydrochloride
297-76-7	Ethynodiol diacetate
298-46-4	Carbamazepine
298-59-9	Methylphenidate hydrochloride
302-79-4	Retinoic acid (tretinoin (all-trans))
303-53-7	Cyclobenzaprine
304-63-2	Erythromycin gluheptonate
306-03-6	Atropine (hyoscyamine hydrobromide)
313-06-4	Estradiol cypionate
338-67-5	Estrogens, conjugated (estrone sodium sulfate)
360-63-4	Betamethasone dihydrogen phosphate
378-44-9	Betamethasone
396-01-0	Triamterene
439-14-5	Diazepam
446-86-6	Azathioprine
469-62-5	Propoxyphene
474-86-2	Estrogens, conjugated (equilin)
481-97-0	Estrogens, conjugated (estrone hydrogen sulfate)
508-96-3	Hydrocortisone tebutate

CAS REGISTRY

NUMBER	COMPOUND
508-99-6	Hydrocortisone cypionate
517-09-9	Estrogens, conjugated (equilenin)
520-85-4	Medroxyprogesterone
549-18-8	Amitriptyline hydrochloride
564-25-0	Doxycycline
573-41-1	Theophylline ethanolamine
620-61-1	Atropine (hyoscyamine sulfate)
630-93-3	Phenytoin sodium salt
643-22-1	Erythromycin stearate
723-46-6	Sulfamethoxazole
738-70-5	Trimethoprim
797-63-7	Norgestrel
797-64-8	Norgestrel ((-) form)
846-49-1	Lorazepam
846-50-4	Temazepam
894-71-3	Nortriptyline hydrochloride
979-32-8	Estradiol valerate
987-24-6	Betamethasone acetate
989-96-8	Triamcinolone 21-(dihydrogen phosphate)
1069-66-5	Valproic acid, sodium salt
1231-93-2	Ethinodiol
1336-20-5	Tetracycline phosphate
1404-04-2	Neomycin
1404-26-8	Polymyxin
1404-90-6	Vancomycin
1404-93-9	Vancomycin hydrochloride
1405-10-3	Neomycin sulfate
1405-12-5	Neomycin palmitate
1405-20-5	Polymyxin B sulfate
1406-04-8	Neomycin undecylenate
1406-11-7	Polymyxin
1420-53-7	Codeine sulfate
1422-07-7	Codeine hydrochloride
1622-61-3	Clonazepam
1639-60-7	Propoxyphene hydrochloride
1997-15-5	Triamcinolone acetone disodium phosphate
2078-54-8	Propofol
2152-44-5	Betamethasone 17-valerate
2203-97-6	Hydrocortisone hemisuccinate
2338-37-6	Propoxyphene (l-form)
2375-03-3	Methylprednisolone sodium succinate
2610-86-8	Warfarin potassium
2921-57-5	Methylprednisolone hemisuccinate
3521-62-8	Erythromycin estolate
3571-53-7	Estradiol undecylenate
3847-29-8	Erythromycin lactobionate
3863-59-0	Hydrocortisone phosphate
4419-39-0	Beclomethasone
4759-48-2	Retinoic acid (isotretinoin (13-cis))
4956-37-0	Estradiol enanthate
4989-94-0	Triamcinolone furetonide

CAS REGISTRY

NUMBER	COMPOUND
5015-36-1	Methylprednisolone sodium phosphate
5534-05-4	Betamethasone acibutate
5534-09-8	Beclomethasone dipropionate
5593-20-4	Betamethasone dipropionate
5600-19-1	Theophylline isopropanolamine
5611-51-8	Triamcinolone hexacetonide
5786-21-0	Clozapine
5913-71-3	Codeine acetate
5928-84-7	Penicillin V benzathine
5967-84-0	Theophylline monohydrate
6000-74-4	Hydrocortisone sodium phosphate
6020-73-1	Codeine salicylate
6069-47-8	Codeine monohydrate
6202-23-9	Cyclobenzaprine hydrochloride
6416-04-2	Tetracycline trihydrate
6493-05-6	Pentoxifylline
6533-00-2	Norgestrel
6533-68-2	Scopolamine hydrobromide trihydrate
6591-72-6	Penicillin V hydrabamine
6835-16-1	Atropine (hyoscyamine sulfate dihydrate)
6854-40-6	Codeine sulfate trihydrate
7177-48-2	Ampicillin trihydrate
8000-10-1	Theophylline sodium glycinate
8002-89-9	Theophylline sodium acetate
8049-62-5	Insulin (zinc suspension)
9002-72-6	Somatropin
9004-10-8	Insulin (injection)
9004-14-2	Insulin (neutral insulin)
9004-17-5	Insulin (protamine zinc suspension)
10238-21-8	Glyburide
11061-68-0	Insulin (human)
11070-73-8	Insulin (cow)
12584-58-6	Insulin (pig)
12629-01-5	Somatropin (human)
12650-69-0	Mupirocin
13311-84-7	Flutamide
13609-67-1	Hydrocortisone butyrate
14838-15-4	Phenylpropanolamine
15307-79-6	Diclofenac sodium
15686-71-2	Cephalexin
15687-27-1	Ibuprofen
15826-37-6	Cromolyn sodium
16110-51-3	Cromolyn
17086-28-1	Doxycycline monohydrate
17140-78-2	Propoxyphene napsylate anhydrous (l-form)
17140-81-7	Nitrofurantoin monohydrate
17693-51-5	Promethazine teoclate
18323-44-9	Clindamycin
18559-94-9	Albuterol
20830-75-5	Digoxin
21256-18-8	Oxaprozin

CAS REGISTRY

NUMBER

COMPOUND

21829-25-4	Nifedipine
22071-15-4	Ketoprofen
22204-53-1	Naproxen
22204-88-2	Tramadol hydrochloride
22254-24-6	Ipratropium bromide
22260-51-1	Bromocriptine mesylate
22298-29-9	Betamethasone benzoate
23067-13-2	Erythromycin gluheptonate
23277-71-6	Ampicillin, potassium salt
23325-78-2	Cephalexin monohydrate
23593-75-1	Clotrimazole
24390-14-5	Doxycycline hyclate
24729-96-2	Clindamycin phosphate
25416-65-3	Levothyroxine sodium hydrate
25507-04-4	Clindamycin palmitate hydrochloride
25614-03-3	Bromocriptine
25812-30-0	Gemfibrozil
26159-34-2	Naproxen, sodium salt
26570-10-5	Propoxyphene napsylate monohydrate
26787-78-0	Amoxicillin
26807-65-8	Indapamide
26839-75-8	Timolol
26921-17-5	Timolol maleate
27203-92-5	Tramadol
28981-97-7	Alprazolam
29094-61-9	Glipizide
29122-68-7	Atenolol
30516-87-1	Zidovudine
31002-79-6	Triamcinolone benetonide
32156-80-2	Theophylline diethanolamine
32388-53-7	Ampicillin monohydrate
33069-62-4	Paclitaxel
33286-22-5	Diltiazem hydrochloride
33386-08-2	Buspirone hydrochloride
33419-42-0	Etoposide
34195-34-1	Hydrocodone bitartrate hydrate
36322-90-4	Piroxicam
36505-84-7	Buspirone
36688-78-5	Clindamycin palmitate
37350-58-6	Metoprolol
41340-25-4	Etodolac
41342-53-4	Erythromycin ethylsuccinate
41444-62-6	Codeine phosphate hemihydrate
41575-94-4	Carboplatin
42399-41-7	Diltiazem
42924-53-8	Nabumetone
50679-08-8	Terfenadine
51022-70-9	Albuterol sulfate
51481-61-9	Cimetidine
53714-56-0	Leuprolide
53994-73-3	Cefaclor

CAS REGISTRY

NUMBER	COMPOUND
54024-22-5	Desogestrel
54182-58-0	Sucralfate
54910-89-3	Fluoxetine
55268-75-2	Cefuroxime
55557-30-7	Propoxyphene napsylate monohydrate (l-form)
56238-63-2	Cefuroxime, sodium salt
56392-17-7	Metoprolol tartrate
56585-33-2	Trimethoprim sulfate
57524-89-7	Hydrocortisone valerate
58001-44-8	Clavulanic acid
58207-19-5	Clindamycin hydrochloride monohydrate
58560-75-1	Ibuprofen (\pm mixture)
59277-89-3	Acyclovir
59333-67-4	Fluoxetine hydrochloride
59467-70-8	Midazolam
59467-94-6	Midazolam maleate
59467-96-8	Midazolam hydrochloride
59865-13-3	Cyclosporine
61054-06-6	Ibuprofen (Al salt)
61177-45-5	Clavulanic acid, potassium salt
61336-70-7	Amoxicillin trihydrate
62571-86-2	Captopril
63074-08-8	Terazosin hydrochloride
63527-52-6	Cefotaxime
63690-57-3	Penicillin V benzathine tetrahydrate
64221-86-9	Imipenem
64336-55-6	Oxycodone terephthalate
64485-93-4	Cefotaxime sodium
64544-07-6	Cefuroxime axetil
65277-42-1	Ketoconazole
65390-64-7	Terazosin
65807-02-5	Goserelin
66357-35-5	Ranitidine
66357-59-3	Ranitidine hydrochloride
66592-87-8	Cefadroxil
66985-17-9	Ipratropium bromide monohydrate
67915-31-5	Terconazole
68373-14-8	Sulbactam
68844-77-9	Astemizole
69388-79-0	Sulbactam pivoxil
69388-84-7	Sulbactam sodium
69657-51-8	Acyclovir, sodium salt
70024-40-7	Terazosin hydrochloride dihydrate
70059-30-2	Cimetidine hydrochloride
70356-03-5	Cefaclor monohydrate
72558-82-8	Ceftazidime
72590-77-3	Hydrocortisone buteprate
73384-59-5	Ceftriaxone
73590-58-6	Omeprazole
74050-20-7	Hydrocortisone aceponate
74103-06-3	Ketorolac

CAS REGISTRY

NUMBER	COMPOUND
74103-07-4	Ketorolac tromethamine
74191-85-8	Doxazosin
74381-53-6	Leuprolide acetate
74431-23-5	Imipenem monohydrate
75330-75-5	Lovastatin
75847-73-3	Enalapril
76095-16-4	Enalapril maleate
76543-88-9	Interferon α A
76547-98-3	Lisinopril
76584-70-8	Valproic acid, semisodium salt
76824-35-6	Famotidine
76963-41-2	Nizatidine
77883-43-3	Doxazosin mesylate
78439-06-2	Ceftazidime pentahydrate
79350-37-1	Cefixime
79559-97-0	Sertraline hydrochloride
79617-96-2	Sertraline
79794-75-5	Loratadine
79902-63-9	Simvastatin
81093-37-0	Pravastatin
81098-60-4	Cisapride
81103-11-9	Clarithromycin
81129-83-1	Cilastatin sodium
81131-70-6	Pravastatin sodium
82009-34-5	Cilastatin
82419-36-1	Ofloxacin
82586-55-8	Quinapril hydrochloride
82626-48-0	Zolpidem
83031-43-0	Sulbactam benzathine
83038-87-3	Doxycycline fosfatex
83784-20-7	Hydrocortisone hemisuccinate monohydrate
83905-01-5	Azithromycin
83915-83-7	Lisinopril dihydrate
83919-23-7	Mometasone furoate
84252-03-9	Erythromycin stinoprate
85056-47-9	Piroxicam olamine
85441-61-8	Quinapril
85721-33-1	Ciprofloxacin
86386-73-4	Fluconazole
86393-32-0	Ciprofloxacin hydrochloride monohydrate
86541-74-4	Benazepril hydrochloride
86541-75-5	Benazepril
87234-24-0	Piroxicam cinnamic acid ester
87333-19-5	Ramipril
88150-42-9	Amlodipine
88150-47-4	Amlodipine maleate
89365-50-4	Salmeterol
90243-99-5	Quinapril hydrochloride monohydrate
90350-40-6	Methylprednisolone suleptanate
91161-71-6	Terbinafine
91524-16-2	Timolol hemihydrate

CAS REGISTRY

NUMBER	COMPOUND
92665-29-7	Cefprozil
93413-69-5	Venlafaxine
93957-55-2	Fluvastatin sodium
96128-89-1	Erythromycin acistrate
98059-61-1	Interferon gamma
98319-26-7	Finasteride
98418-47-4	Metoprolol succinate
99210-65-8	Interferon α 2B
99294-93-6	Zolpidem (+)-tartrate (2:1)
99300-78-4	Venlafaxine hydrochloride
99614-01-4	Ondansetron hydrochloride dihydrate
99614-02-5	Ondansetron
100680-33-9	Cefuroxime pivoxetil
103628-46-2	Sumatriptan
103628-48-4	Sumatriptan succinate
103639-04-9	Ondansetron hydrochloride dihydrate
104376-79-6	Ceftriaxone sodium
105857-23-6	Alteplase
105879-42-3	Cephalexin hydrochloride
111470-99-6	Amlodipine besylate
112017-99-9	Ibuprofen (piconol)
113427-24-0	Epoetin alfa
116002-70-1	Ondansetron
117091-64-2	Etoposide phosphate
119637-66-0	Metoprolol fumarate
121123-17-9	Cefprozil monohydrate
121961-22-6	Loracarbef monohydrate
122312-54-3	Epoetin beta

CROSS REFERENCE TO *THE MERCK INDEX*

For each drug the relevant abstract number in the 12th edition of *The Merck Index* (Budavari, S., Ed., *The Merck Index*, 12th edition, Merck & Co. Inc.: Whitehouse Station, NJ, 1996) is given. Much useful information, such as melting point, solubility, optical rotation, and references to reviews, can be found in *The Merck Index*.

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CROSS REFERENCE TO THE ORGANIC CHEMISTRY OF DRUG SYNTHESIS

For each drug the relevant sections in the series *The Organic Chemistry of Drug Synthesis* by Lednicer *et al.* are identified. The volume number is followed by the page number. This series gives valuable information about the syntheses of various drugs, and this may be helpful in determining impurities, understanding degradation reactions, and so on. The volumes in this series are:

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